Laboratory for Molecular Psychosomatics
Department of Psychosomatic Medicine and Psychotherapy
Head of Clinic: Prof. Dr. med. Harald Gündel

Investigation of the protective effects of *Mycobacterium vaccae* ATCC 15483^{T} against the negative consequences of early life adversity or chronic psychosocial stress during adulthood.

Dissertation submitted in partial fulfilment of the requirements for the degree of "Doctor rerum naturalium" (Dr. rer. nat.) of the International Graduate School in Molecular Medicine Ulm

Jessica Schiele
born in Heidenheim an der Brenz, Germany

2026

1. **Current dean of the medical faculty:** Prof. Dr. Thomas Wirth

2. **Current chairman of the Graduate School:** Prof. Dr. Bernd Knöll

3. **Thesis Advisory Committee:**

    - First supervisor: Prof. Dr. Stefan O. Reber (Ulm, Germany)
    - Second supervisor: Univ.-Prof. Dr. Melanie Haffner-Luntzer (Ulm, Germany)
    - Third supervisor: Prof. Dr. Christopher A. Lowry (Boulder, USA)

4. **Day doctorate awarded:** June 30th, 2026

Results obtained in my thesis have previously been published in the following publication:

Schiele J*, Tsai PL*, Schimmele T, Beck S, Meyer M, Mannes M, Desmond LW, Noschka R, Huber-Lang M, Haffner-Luntzer M, Jarczok MN, Lowry CA, Reif A, Langgartner D, Stenger S, Slattery DA*, Reber SO*, 2026

Microbial Legacy: *Mycobacterium vaccae* ATCC 15483^{T} intergenerationally diversifies the microbiome and enhances stress resilience in male mice.

Molecular Psychiatry

doi: 10.1038/s41380-026-03638-9

Schiele J*, Tsai PL*, Ernst G, Blank C, Beck S, Schimmele T, Burth E, Gebauer D, Lowry CA, Noschka R, Langgartner D, Stenger S, Haffner-Luntzer M, Slattery DA*, Reber SO*, 2026 (Epub 2025)

Mycobacterium vaccae ATCC 15483^{T} administered to nulliparous C57BL/6N mice prior to mating protects their male offspring against the negative consequences of prenatal stress.

Brain, Behavior, and Immunity

doi: 10.1016/j.bbi.2025.106169

Table of Contents

ABBREVIATIONS **3**

INTRODUCTION **6**

Increased immune activation in stress-associated affective disorders 9

The CSC paradigm as a preclinical model for chronic psychosocial stress in adult male mice 10

The SIP as a preclinical model for chronic psychosocial stress in adult female mice 16

Chronic stress induces microbiome disruption 17

Prenatal stress as driver for long-term health risks 21

"Old Friends" hypothesis 23

The immunoregulatory nature of *Mycobacterium vaccae* 26

Aims of this thesis 31

MATERIALS AND METHODS **33**

Materials 33

Methods 38

RESULTS **65**

Intergenerational effects of *M. vaccae* ATCC 15483^T on the negative behavioral, physiological and immunological consequences of chronic psychosocial stress in adult C57BL/6N male and female offspring 65

***M. vaccae* ATCC 15483^T intergenerationally diversifies the microbiome of male offspring and increases the relative abundance of potent SCFA-producing taxa** 76

Effects of *M. vaccae* ATCC 15483^T on litter characteristics and maternal behavior 82

Effects of *M. vaccae* ATCC 15483^T and prenatal stress on behaviors, physiology, immunology, and bone metabolism in male and female offspring 85

Effects of *M. vaccae* ATCC 15483^T and prenatal stress on behaviors, physiology, immunology, and bone metabolism in single-housed adult male offspring 91

Effects of *M. vaccae* ATCC 15483^T and prenatal stress on immunology in male pups 95

Effects of *M. vaccae* ATCC 15483^T and prenatal stress on litter characteristics and maternal behavior 96

DISCUSSION **98**

Preconceptional maternal treatment with *M. vaccae* ATCC 15483^{T} enhances stress resilience and microbiome diversity in adult male offspring 99

Preconceptional *M. vaccae* ATCC 15483^{T} treatment promotes intergenerational resilience in prenatal stress-exposed male offspring 106

Conclusions 109

SUMMARY **112**

LIST OF REFERENCES **114**

LIST OF PUBLICATIONS **148**

ACKNOWLEDGMENTS **151**

STATUTORY DECLARATION **153**

Abbreviations

ACTH	adrenocorticotropic hormone	CRP	C-reactive protein
ANOVA	analysis of variance	CSC	Chronic subordinate colony housing
ASV	amplicon sequence variant	DC	dendritic cell
ATCC	American Type Culture Collection	DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
AVP	arginine vasopressin	DSS	dextran sulphate sodium
BBS	borate-buffered saline	ELA	early life adversity
BCA	bicinchoninic acid	ELISA	Enzyme-linked immuno-precipitation assay
BCECF	2',7'-bis-(2-carboxyethyl)-5-(6)-carboxyfluorescein	EPM	elevated plus maze
BM	bone marrow	F/B	Firmicutes/Bacteroidetes
bp	base pair	FACS	flow-associated cell sorting
BrdU	bromodeoxyuridine	FCS	fetal calf serum
BSA	bovine serum albumin	FoxP3	forkhead box protein p3
BV/TV	bone volume to tissue volume ratio	FST	forced swim test
c	cortical	GC	glucocorticoid
cAMP	cyclic adenosine monophosphate	GHC	group-housed control
CA	closed arm	GR	glucocorticoid receptor
CAS	chronic adult stress	HA	hydroxyapatite
CD	cluster of differentiation	HBSS	Hanks' balanced salt solution
CECT	Colección Española de Cultivos Tipo	HPA	hypothalamic-pituitary-adrenal
CFSE	Carboxyfluorescein succinimidyl ester	*H. polygyrus*	*Heligmosomoides polygyrus*
CORT	corticosterone		
CRH	corticotropin-releasing hormone		

IBD	inflammatory bowel disease	NaCl	Sodium chloride/saline
i.d.	intradermal	NCTC	National Collection of Type Cultures
IFNγ	interferon gamma	NTM	non-tuberculous mycobacteria
i.g.	intragastric	OA	open arm
Ig	immunoglobulin	OD	optical density
IL	interleukin	OF/NO	open field/novel object
i.n.	intranasal		
i.p.	intraperitoneal	PBMC	peripheral blood mononuclear cells
IS	inescapable tail shock	PBS	phosphate-buffered saline
LBP	LPS-binding protein	PCR	polymerase chain reaction
LFC	log fold change	PD	pregnancy day
LMM	Linear-Mixed-Model	PMN	polymorphonuclear
LPS	lipopolysaccharide	PPAR	peroxisome proliferator-activated receptor
Ly6C or Ly6G	lymphocyte antigen 6 complex, locus C or G	PRR	pattern recognition receptor
MAMP	microbial-associated molecular pattern	PRT	pup retrieval test
MCP	Monocyte chemotactic protein	PS	prenatal stress
MDD	major depression disorder	PTSD	posttraumatic stress disorder
MDSC	myeloid-derived suppressor cell	RANKL	receptor activator of NF-κB ligand
mesLNC	mesenteric lymph node cell	RC	red color
MO	monocytes/monocytes-like	RGM	rapid growing mycobacteria
MS	maternal separation	RORγt	Retinoic acid-related orphan receptor gamma t
M. vaccae	*Mycobacterium vaccae*	RSDS	repeated social defeat stress
MWU	Mann-Whitney U	RST	restraint stress test

s.c.	subcutaneous
SCFA	short-chain fatty acid
SEM	standard error of the mean
SENS	sensory
SHC	single-housed control
sIgA	secretory IgA
SIP	social instability paradigm
SPAT	social preference/avoidance test
SPS	single prolonged stress
SPT	sucrose or saccharin preference test
T	Typestrain
Tb	trabecular
TbN	trabecular number
TbSp	trabecular separation
TBS-T	Tris-buffered saline containing Tween
TbTh	trabecular thickness
TbTMD	trabecular tissue mineral density
Th	T helper
TH	tyrosine hydroxylase
TLR	toll-like receptor
TMD	tissue mineral density
TNF	tumor necrosis factor
Treg	regulatory T cell
TSST	Trier Social Stress Test
Veh	vehicle
VOI	volume of interest
WBC	white blood cell
ZO	zonula occludens

Introduction

Chronic psychosocial stress, as major burden in modern society, represents a risk factor for somatic and mental disorders, such as cardiovascular diseases (Buckley et al., 2012; Undén et al., 1991), carcinogenesis (Peters et al., 2012), fibromyalgia (Almansa et al., 2009), bronchial asthma (Wright et al., 2004), rheumatoid arthritis and lupus erythematosus (Richter et al., 2018), inflammatory bowel disease (IBD) (Podolsky, 2002; Reber, 2012; Robertson et al., 1989), stomach ulcers (Coker et al., 2000), as well as anxiety disorders (Moura et al., 2022), depression (Agid et al., 1999; de Kloet et al., 2005; Pace et al., 2006), posttraumatic stress disorder (PTSD) (Gola et al., 2013; Lindqvist et al., 2014; Reber et al., 2016a; Rohleder, 2014) and chronic fatigue syndrome (Heim et al., 2009) in later life. Importantly, psychosocial stressors are not limited to adulthood (chronic adult stress; CAS), but can occur across multiple life stages, e.g., during early life (Danese and McEwen, 2012), puberty and/or adolescence. While early life adversities (ELAs) occurring before birth are referred to as prenatal stress (PS), ELAs occurring after birth during (early) childhood are referred to as postnatal stress. Interestingly, according to the multiple-hit concept (Daskalakis et al., 2013), ELA is thought to exaggerate the negative impact of CAS, while according to the match-mismatch hypothesis (Nederhof and Schmidt, 2012; Santarelli et al., 2017; Schmidt, 2011), ELA is preparing an individual to better cope with CAS.

Although the mechanisms underlying stress-associated disorders are not fully understood until today, most of the aforementioned stress-associated diseases are accompanied by an over-(re)active immune system and chronic low-grade inflammation (Gola et al., 2013; Habib et al., 2022; Howren et al., 2009; Michopoulos et al., 2017; Pearson et al., 2003; Podolsky, 2002; Rohleder, 2014). Moreover, several prospective human and mechanistic animal studies support the hypothesis that a failure in immunoregulation, due to a compromised regulatory T (Treg) cell compartment (Li et al., 2010; Sommershof et al., 2009), plays a causal role in the pathogenesis of stress-related somatic and mental disorders (Covic et al., 2012; Dantzer, 2017; Duivis et al., 2011; Hodes et al., 2014; Khandaker et al., 2014; Kivimaki et al., 2014; Pace et al., 2006).

Epidemiological data further indicate that the prevalence of stress-associated somatic and psychiatric disorders has steadily increased in recent decades (Alonso et al., 2011; Bach, 2002; Lu et al., 2024; Wang et al., 2024b; Woolcock and Peat, 1997; Zhang et al., 2023), with men being more vulnerable for cancer (Jackson et al., 2022), autism spectrum disorder (Tusa et al., 2025), and externalizing psychiatric symptoms, including substance abuse and

aggression (Eaton et al., 2012; Kuehner, 2017), while women are more vulnerable for multiple sclerosis (Walton et al., 2020), fibromyalgia (Ruschak et al., 2023), rheumatoid arthritis and lupus erythematosus (Finckh et al., 2022; Gergianaki et al., 2018), bronchial asthma (Borrelli et al., 2025), IBD (Tshikudi et al., 2025), and internalizing psychiatric diseases such as anxiety disorders, depression and PTSD (Bangasser and Valentino, 2014; Kessler, 2003; Kessler et al., 1993; Kuehner, 2017).

In addition to these profound sex differences, the environment in which an individual grows up also appears to influence the prevalence of stress-associated mental and affective disorders (Peen et al., 2010; Xu et al., 2023b). In line with the latter and the hypothesis that an overshooting and uncontrolled inflammatory stress response is a risk factor for developing stress-associated somatic and affective disorders, individuals raised in urban versus rural areas show a more pronounced immune activation in response to a standard laboratory stressor, namely the Trier Social Stress Test (TSST) (Böbel et al., 2018). One mechanism underlying this immunoregulatory deficit in urban versus rural individuals might be the reduced exposure to immunoregulatory microbes with which humans and other mammals co-evolved ("Old Friends"). This is particularly pronounced in highly-industrialized modern societies (Blaser, 2017; Blaser and Falkow, 2009; Langgartner et al., 2025; Pfefferle et al., 2021; Strachan, 1989, 2000). These so-called "Old Friends" promote immunoregulation by inducing Tregs and stimulating the secretion of the antiinflammatory cytokine interleukin (IL-)10 (Langgartner et al., 2019; Rook et al., 2013a). Of particular importance in the context of the current thesis, prenatal (Ege et al., 2008; Schaub et al., 2009), but also early postnatal (Riedler et al., 2001), exposure to immunoregulatory microorganisms seems to promote immunoregulation and protect against stress-associated diseases (McDade et al., 2013; Mishra et al., 2021; Rook, 2013; Rook et al., 2013b).

Therefore, bringing back "Old Friends" might represent an innovative strategy to attenuate stress-induced inflammation and thereby promote stress resilience. In line with the latter, previous publications have recently shown that subcutaneous (s.c.) administrations of a heat-killed preparation of *Mycobacterium (M.) vaccae* NCTC (National Collection of Type Cultures) 11659 during adulthood is effective in stabilizing the gut microbiome (Foxx et al., 2020; Reber et al., 2016b), increasing the percentage of Treg within mesenteric lymph node cells (mesLNCs) (Reber et al., 2016b), preventing stress-induced colitis and *ex vivo* pro-inflammatory cytokine secretion from anti-cluster of differentiation (CD)3-stimulated mesLNCs (Reber et al., 2016b), preventing stress-induced exaggeration of anxiety (Amoroso et al., 2020a; Frank et al., 2018a; Loupy et al., 2021; Reber et al., 2016b), as well as microglial

priming and neuroinflammation (Fonken et al., 2018a; Fonken et al., 2018b; Frank et al., 2018a; Frank et al., 2018b). Moreover, when administered during adolescence *M. vaccae* NCTC 11659 prevents maternal separation (MS)-induced impairment of bone metabolism (Gebauer et al., 2025; Schimmele et al., 2025). Importantly, not only *M. vaccae* NCTC 11659 but also the closely related strain *M. vaccae* ATCC (American Type Culture Collection) 15483$^{\text{Typestrain(T)}}$ has immunoregulatory and stress-protective effects when administered repeatedly *via* the s.c. route (Desmond et al., 2024; Loupy et al., 2021).

Based on what I have reported above, this thesis aims to investigate whether repeated, less invasive intragastric (i.g.) administration of the heat-inactivated, non-pathogenic *M. vaccae* ATCC 15483^{T} to nulliparous C57BL/6N female mice prior to mating can intergenerationally ameliorate the negative behavioral, physiological, and immunological consequences of chronic psychosocial stress experienced either prenatally or during adulthood in their male and female offspring.

Increased immune activation in stress-associated affective disorders

A common aspect of chronic stress-associated affective disorders, such as depression and PTSD, is an over-(re)active immune system and chronic low-grade inflammation (Gola et al., 2013; Iwata et al., 2013; Kivimaki et al., 2014; Lindqvist et al., 2014; Miller and Raison, 2016; Reber, 2012). For instance, patients with depression exhibit an immune cell profile characterized by increased numbers of white blood cells (WBCs), neutrophils, and monocytes, as well as elevated counts of class II major histocompatibility complex human leukocyte antigen determinant region-bearing cells, memory T cells, and IL-2 receptor-expressing cells, indicating systemic immune activation (Maes et al., 1992). Moreover, patients with major depressive disorder (MDD) display enhanced *ex vivo* secretion of IL-1β (Maes, 1995), elevated plasma concentrations of IL-6 (Pace et al., 2006), tumor necrosis factor (TNF) and C-reactive protein (CRP) (Miller et al., 2009), as well as increased *ex vivo* IL-6 production in the supernatant of mitogen-stimulated peripheral leukocytes (Maes et al., 1993). Additionally, correlation analysis between IL-6 levels in the supernatant of mitogen-stimulated peripheral leukocytes and the post-dexamethasone cortisol concentrations, dexamethasone is a synthetic glucocorticoid (GC) analogue, revealed positive associations, suggesting the development of GC resistance in depressed individuals (Maes et al., 1993). This hypothesis is supported by findings showing that GCs inhibit IL-6 production in monocytes by interfering with IL-6 gene transcription (Zanker et al., 1990). Reviews by Maes (1999), Miller et al. (2009), and Miller and Raison (2016) provide additional evidence supporting the presence of an enhanced inflammatory response in patients with MDD. In detail, hallmark features of this inflammatory response include increased levels of cytokines and their respective receptors, acute-phase proteins, chemokines, and soluble adhesion molecules in peripheral blood as well as the cerebrospinal fluid. In postmortem brain samples of suicide victims who suffered from depression, elevated expression of genes and proteins associated with the innate immune system, such as IL-1β, IL-6, TNF, toll-like receptor (TLR)3, and TLR4 has also been observed (Miller and Raison, 2016). Furthermore, gene expression profiles of peripheral blood confirm a pro-inflammatory, so-called “M1” macrophage phenotype. In addition, polymorphisms in inflammatory cytokine genes encoding IL-1β, TNF and CRP have been linked to the development of depression and the variability in treatment response (Miller and Raison, 2016).

As mentioned above, stressor- and trauma-related disorders, such as PTSD, are also accompanied by an over(re-)active immune system, and inflammation has been associated with the vulnerability to and the persistence of PTSD symptoms.

A clinical study reported increased nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway activity in women with PTSD resulting from childhood abuse, which was positively correlated with both the severity of PTSD symptoms and reduced GC sensitivity in whole-blood monocytes in comparison to healthy controls (Pace et al., 2012). Moreover, isolated peripheral blood mononuclear cells (PBMCs) of PTSD patients produced significantly higher amounts of pro-inflammatory IL-1β, IL-6, and TNF than PBMCs of the control group (Gola et al., 2013). This is further supported by high serum levels of proinflammatory cytokines in male soldiers diagnosed with military combat-related PTSD, even when depression and early life trauma was taken into account (Lindqvist et al., 2014). Posttraumatic stress can also be associated with the exacerbation of inflammatory disorders, such as cardiovascular events (von Känel et al., 2010) or IBD (Cámara et al., 2011), with increased levels of proinflammatory biomarkers, including CRP and TNF, playing an important role (Spitzer et al., 2010; Vermeire et al., 2004).

Despite the significant progress in stress research and the identification of several important underlying mechanisms, many aspects of chronic psychosocial stress and its contribution to the development of affective disorders in humans remain poorly understood. To further investigate these mechanisms, animal models have been developed. Such a preclinical model is the well-established chronic subordinate colony housing (CSC) paradigm, a validated rodent model for chronic psychosocial stress and PTSD (Langgartner et al., 2015; Nyuyki et al., 2012; Reber et al., 2016a; Reber and Neumann, 2008; Slattery et al., 2012). The following section outlines the experimental setup of the CSC paradigm, its characteristics, as well as recent insights that might contribute to the development of novel treatment strategies.

The CSC paradigm as a preclinical model for chronic psychosocial stress in adult male mice

The CSC paradigm relies on the establishment of a social hierarchy resulting from repeated confrontations between a dominant, more aggressive CD-1 male mouse, the resident of the experimental cage, and four experimental C57BL/6N male mice, the intruders (Amoroso et al., 2020a; Amoroso et al., 2019; Amoroso et al., 2020b; Foertsch and Reber, 2020; Langgartner et al., 2015; Mazzari et al., 2023; Reber et al., 2007; Reber et al., 2016a; Reber and Neumann, 2008; Reber et al., 2016b; Slattery et al., 2012). To prevent habituation to the established social hierarchy, experimental CSC mice are exposed to a novel resident mouse on day 8 and day 15. Prior to CSC, CD-1 males are tested for their aggressiveness by brief group housing with five conspecifics.

CD-1 male mice that fail to attack or severely injure their opponents are excluded from the use in the CSC paradigm, whereas males showing dominant behavior without severe biting are selected (Langgartner et al., 2015; Reber et al., 2007). This approach strongly reduces the number of bite wounds. However, it does not completely prevent them (Foertsch and Reber, 2020; Langgartner et al., 2015; Reber et al., 2016a). Depending on the experimental setup, including the performance of one or multiple behavioral tests before the end of CSC, experimental mice are euthanized by rapid decapitation following brief CO_2 exposure in the morning after the final behavioral assessment, between day 19 and day 22 (Amoroso et al., 2020a; Foertsch et al., 2017a; Kempter et al., 2023; Langgartner et al., 2023a; Mazzari et al., 2023). Previous studies have demonstrated that undisturbed, same-age single-housed controls (SHCs) are adequate controls, as group housing conditions *per se* were found to be stressful in C57BL/6N males, as group-housed and CSC males exhibited comparable levels of anxiety when compared with SHCs (Busch et al., 2013; Reber et al., 2016a; Singewald et al., 2009).

Consistent with the characteristics of stress-associated affective disorders, chronic psychosocial stress in the mouse model leads to the activation of the immune system, reflected by elevated plasma levels of proinflammatory cytokines such as IL-1β and IL-6, as well as granulocyte colony stimulating factor and monocyte chemotactic protein-1 (MCP-1), indicating systemic inflammation (Langgartner et al., 2018b). Moreover, it has been extensively discussed that the stress-induced immune system (over)activation is mediated by changes within the innate compartment, particularly affecting myeloid cells (Hodes et al., 2014; Kivimaki et al., 2014). In line with the latter, $CD11b^+$ myeloid subsets, such as neutrophils and monocytes, have been identified as contributors to the immunological consequences following chronic psychosocial stress in CSC mice (Foertsch et al., 2017a).

To better understand why these cells are of particular importance, a brief overview of the hypothalamus-pituitary-adrenal (HPA) axis and the GC signaling is warranted. An acute stressor triggers the HPA axis to secrete corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from parvocellular neurons of the hypothalamic paraventricular nucleus into the portal blood stream to the pituitary. Both CRH and AVP stimulate the synthesis and release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the peripheral circulation. In turn, ACTH induces the production of GCs (cortisol in humans, corticosterone (CORT) in rodents) in the cortical cells of the adrenal gland and the release into the bloodstream. The activation of the HPA axis enables rapid mobilization of glucose and oxygen, metabolites essential for adaptation and survival (Langgartner et al., 2015).

Binding of GCs to their respective receptors, namely glucocorticoid (GR) or mineralocorticoid receptors at different levels of the HPA axis mediates the negative feedback inhibition, thereby terminating the acute HPA axis response (Reul and de Kloet, 1985).

In the context of chronic activation of the HPA axis, a common feature of stress-induced somatic and affective disorders (Agid et al., 1999; de Kloet et al., 2006; de Kloet et al., 2005; Dimsdale, 2008; Robertson et al., 1989; Wright et al., 2004), altered and impaired GC signaling plays a critical role. This impairment may result from decreased hormone bioavailability, GC insensitivity or enhanced resistance of targeted immune cells (Raison and Miller, 2003).

The CSC paradigm represents a powerful experimental tool with high face validity, combining chronic, psychological and social aspects of stress that mimic health-compromising stressors faced by humans, as it induces reduced GC signaling in the experimental mice (Langgartner et al., 2015; Reber et al., 2016a). However, it should be noted that, in CSC mice, the development of GC resistance depends on bite injuries received during physical confrontations with the resident mouse (Foertsch et al., 2017a).

Taking the severity of these bite wounds into account, a recent preclinical study employing the CSC model identified the $CD11b^+$ lymphocyte antigen 6 complex, locus G $(Ly6G)^+$ lymphocyte antigen 6 complex, locus C $(Ly6C)^+$ polymorphonuclear (PMN)-myeloid-derived suppressor cells (MDSCs) as a key immune cell population for stress-induced GC resistance (Kempter et al., 2023). A possible explanation for the underlying mechanism of GC insensitivity could be that exposure to psychosocial stressors primes and activates PMN-MDSCs, neutrophils, and monocyte/monocyte-like (MO)-MDSCs locally in the bone marrow (BM), as indicated by an upregulation of TLR2 and by the increased basal and lipopolysaccharide (LPS; a surface marker of gram negative bacteria)-induced *ex vivo* cell viability of myeloid BM cells (Kempter et al., 2023). Subsequently, these primed and activated myeloid cells migrate *via* the peripheral circulation to the spleen, where they accumulate and contribute to splenomegaly in response to coinciding physical injury and translocated intestinal microbiota (Allen et al., 2012; Lafuse et al., 2017). Moreover, both splenic PMN-MDSCs and monocyte/MO-MDSCs exhibit an upregulation of TLR4. However, LPS-induced *ex vivo* functional splenic GC resistance was detectable exclusively in isolated $Ly6G^+$ PMN-MDSCs, but not in $Ly6G^-$ monocyte/MO-MDSCs. Correlation analysis further revealed that TLR4 expression on PMN-MDSCs plays a critical role in CSC-induced *ex vivo* GC resistance (Kempter et al., 2023). This upregulation of TLR4 is proposed to trigger a shift towards the proinflammatory NF-κB signaling pathway upon LPS-stimulation (Avitsur et al., 2003;

Kempter et al., 2023). In turn, enhanced NF-κB signaling exceeds the antiinflammatory capacity of GCs, which is associated with compromised GR function, characterized by impaired nuclear translocation and a lack of transcriptional suppression in isolated splenocytes from socially stressed mice (Foertsch et al., 2017a; Kempter et al., 2021; Quan et al., 2003; Reber et al., 2007).

Further insights demonstrated that GC insensitivity of $CD11b^+$ splenocytes can also occur following sterile abdominal surgeries, e.g., an E-Mitter implantation, indicating that sterile wounding in combination with chronic stress also contributes to GC resistance even in the absence of visible bite wounds (Foertsch et al., 2020; Foertsch and Reber, 2020).

Moreover, in support of the hypothesis that a decreased immunoregulation mediated by a substantial reduction in Tregs is likely to play a role in stress-associated psychiatric disorders (Sommershof et al., 2009), CSC exposure in mice was also associated with reduced Treg counts in both peripheral lymph nodes (Schmidt et al., 2016) and the spleen (Langgartner et al., 2023a). Tregs are defined by the expression of CD4 and CD25, markers indicative for their lineage, and the transcription factor forkhead box protein p3 (FoxP3), which is essential for the differentiation of naïve T cells into Tregs and for the acquisition of their suppressive phenotype (Fontenot et al., 2003; Hori et al., 2003). The concept of Treg-dependent immune dysregulation in chronic inflammatory psychiatric disorders has led to the suggestion that Treg stimulation might represent a promising therapeutic strategy (Ellul et al., 2018), which will be later introduced in the chapters "*Old Friends hypothesis*" and "*The immunoregulatory nature of* Mycobacterium vaccae*"*.

Chronic psychosocial stress not only induces changes on the level of the immune system of experimental mice, but also shows profound consequences on a behavioral level in comparison to SHC mice. For instance, using the social preference/avoidance test (SPAT), Amoroso et al. (2020a; 2019) found that chronically traumatized experimental mice show a lack of social preference, which is in line with evidence that individuals suffering from PTSD present comorbid social anxiety disorders (Gros et al., 2023). Moreover, long-lasting stress-induced hyperarousal or hyperactivity, another hallmark of PTSD in humans (Yehuda, 2002), typically represented by increased locomotion has been observed in experimental animals one week after CSC exposure (Slattery et al., 2012). By testing CSC mice versus their respective controls for general anxiety-related behavior using the open field/novel object (OF/NO) test, elevated plus maze (EPM), SPAT, the light-dark box test, and the elevated

platform exposure (Langgartner et al., 2015; Reber et al., 2016a), increased anxiety-like behavior was observed in CSC males. Increased general anxiety is also a well-established indicator of chronic psychosocial stress in humans (Cryan and Holmes, 2005).

Furthermore, CSC exposure induces physiological alterations such as the reduction in thymus weight (Reber et al., 2007) as well as an increase in pituitary and adrenal weight (Füchsl et al., 2013). These changes in stress-sensitive organs are well-known predictive biomarkers of chronic stress (Langgartner et al., 2018a). Besides the altered morphology, adrenal glands of CSC mice versus SHCs were thought to show a compromised functionality, as indicated by significantly decreased CORT concentrations released in response to *ex vivo* ACTH stimulation, a phenomenon at that time termed as adrenal insufficiency (Reber et al., 2007). However, elevated plasma CORT levels were detected after behavioral testing, indicating an increased HPA reactivity in CSC mice when exposed to unfamiliar, novel stressors (Uschold-Schmidt et al., 2012).

Given the comorbidity of PTSD and IBD in humans (Mawdsley and Rampton, 2005), these observations are in line with the findings in CSC mice which show spontaneous development of colonic inflammation (Reber et al., 2007; Reber et al., 2011). Colonic inflammation is characterized by increased 1) histological damage of colon tissue, 2) intestinal permeability followed by enhanced bacterial translocation, 3) colonic macrophages, dendritic and T cell counts, and 4) secretion of cytokines including interferon gamma (IFNγ), IL-6, IL-10, IL-13, IL-17, and TNFα from isolated and *ex vivo* stimulated mesLNCs (Reber et al., 2008). Additionally, dextran sulphate sodium (DSS)-induced colitis was found to be exacerbated by the exposure to CSC (Langgartner et al., 2024; Reber et al., 2008).

Moreover, there is evidence for a bidirectional relationship between stress-induced depression and metabolic bone disorders such as osteoporosis (Cizza et al., 2010; Kashfi et al., 2022). This is most likely due to shared increased inflammatory mediators (Saxena et al., 2021). However, the underlying mechanisms remain unclear. Experiments employing the CSC paradigm have shown that chronic psychosocial stress is associated with impaired endochondral ossification in the growth plate of long bones, resulting in reduced tibia and femur lengths (Foertsch et al., 2017b). Furthermore, CSC-exposed mice exhibit increased trabecular thickness, elevated bone mineral density and enhanced tyrosine hydroxylase (TH) expression in BM cells located within the growth plate (Foertsch et al., 2017b). Supporting the hypothesis that local catecholaminergic signaling contributes to the stress-induced skeletal alterations, Tschaffon-Müller et al. (2023) demonstrated that depletion of TH in $CD11b^{+}$

BM cells of CSC mice prevented stress-induced disturbances in bone growth and regeneration. Of further importance for understanding the sequelae of chronic psychosocial stress exposure is the fact that trauma is not limited to individuals directly experiencing severe stressors. Indirect exposure, for instance by witnessing the traumatization of others, can also trigger the development of PTSD symptoms (Flannery, 2022; Orrù et al., 2021). This aspect highlights that the stress response is not solely dependent on direct physical or psychosocial stress, but can also be initiated by observation or empathy. In support of this theory, experimental human studies employing the TSST have reported that merely observing individuals undergoing direct acute stress exposure can elicit a physiological stress response, including elevated levels of endogenous cortisol, in the observers (Buchanan et al., 2012). These findings are further supported by preclinical models in which experimental rodents are maintained in sensory (SENS) contact with their conspecifics exposed to stressful physical confrontations and also exhibit robust stress responses. These SENS animals showed elevated plasma CORT concentrations, along with typical physiological stress markers such as adrenal hypertrophy and thymus involution (Finnell et al., 2017; Langgartner et al., 2018a; Veenema et al., 2005). Moreover, mice kept in sensory contact with the final dominant CD-1 resident even after direct chronic stressor exposure displayed delayed recovery from stress-induced disruption of circadian rhythm compared to CSC controls without post-exposure contact. This finding indicates that ongoing sensory exposure to a known aggressor can profoundly impair stress recovery in observers (Foertsch et al., 2019).

Due to its experimental design, the CSC paradigm can only be used to investigate chronic psychosocial stress in male rodents. Over the past decades, most preclinical studies have likewise relied on male laboratory animals (Zucker and Beery, 2010). However, both epidemiological and experimental studies have revealed that the prevalence of stress-associated affective disorders is sex-specific (Breslau, 2009; Hodes and Epperson, 2019), given that psychiatric disorders such as depression and PTSD are more common in women than in men (Bangasser and Valentino, 2014; Kuehner, 2017). These sex differences suggest that hormonal influences play a role in modulating the physiological stress response (Kirschbaum et al., 1999). Consequently, increasing attention has been directed toward stress research in females (Kajantie and Phillips, 2006; Oyola and Handa, 2017; van Doeselaar et al., 2021). The next section outlines the establishment of a preclinical stress model, i.e., the social instability paradigm (SIP), that is applicable to female mice.

The SIP as a preclinical model for chronic psychosocial stress in adult female mice

Back in 1999, Haller and colleagues highlighted the lack of experimental animal models suitable for studying stress in females (Haller et al., 1999). They found that social defeat is more effective in males, likely due to the stronger dominance hierarchies among males, while females are more vulnerable to social instability stress in the absence of obvious aggression (Haller et al., 1999). Nevertheless, Sterlemann et al. (2008) showed that the seven-weeks SIP, involving group composition changes every third day using randomized rotation schedules to minimize repeated interactions with the same individuals (Schmidt et al., 2007), successfully induced typical stress-related alterations in male mice, such as increased adrenal weight, decreased thymus weight, disrupted circadian levels of CORT and ACTH, and heightened anxiety-like behaviors. Notably, some of these effects persisted up to 12 months after stress exposure, indicating long-term behavioral and neuroendocrine consequences (Sterlemann et al., 2008). It was previously demonstrated that "social instability is an effective chronic stress paradigm for both male and female mice" (Yohn et al., 2019). Accordingly, negative consequences observed in women suffering from stress-associated mental disorders are mirrored by SIP-exposed female rodents, as evidenced by increased general (Jarcho et al., 2016; Schmidt et al., 2010) and social (Baranyi et al., 2005) anxiety-like behaviors, as well as chronic activation of the HPA axis indicated by elevated plasma CORT levels when compared with respective undisturbed group-housed controls (GHCs) (Schmidt et al., 2010). Moreover, preclinical physiological biomarkers of chronic stress, such as adrenal hypertrophy and thymus involution (Langgartner et al., 2018a), have been also identified in females subjected to the SIP (Schmidt et al., 2010).

In contrast to the studies in which these physiological findings were obtained, a previous experiment using the SIP conducted in our group was unable to reproduce the expected stress-induced behavioral and physiological changes (Mazzari et al., 2023). This discrepancy might be attributed to stressful handling procedures during the administration of an immunoregulatory agent prior to stress exposure (Mazzari et al., 2023). Nevertheless, Mazzari et al. (2023) reported increased levels of proinflammatory cytokine IL-4, elevated numbers of splenic myeloid cells and PMN-MDSCs, and a reduction in spleen weight, contrasting with splenomegaly typically observed in physically injured male mice. Consequently, the authors suggested that myeloid immune cells emigrate from the spleen in response to CAS (Mazzari et al., 2023).

Furthermore, unlike in CSC exposed males, the increased number of CD11b and Ly6G expressing neutrophils was not associated with enhanced *ex vivo* viability of splenocytes indicating that stress-induced priming and activation might not have occurred at the BM level (Mazzari et al., 2023). Given that female mice typically exhibit flat social hierarchy and low dominance behavior, consequences associated with wounding, such as splenomegaly and splenic *ex vivo* functional GC insensitivity, are likely not detectable in females (Mazzari et al., 2023).

Beyond its negative impact on behavior, physiology, and immunology reported in both male and female mice, chronic psychosocial stress has additionally been shown to profoundly change the gut microbiome, a factor increasingly recognized as contributing to the development of stress-associated mental disorders and serving as a key link to chronic inflammatory diseases, such as IBD (Mawdsley and Rampton, 2005), which is frequently comorbid with depression or PTSD. In the following section, this topic will be outlined in detail.

Chronic stress induces microbiome disruption

Chronic psychosocial stress is strongly linked to disruptions in gut microbial composition and increased intestinal barrier permeability (Dinan and Cryan, 2017; Forsythe et al., 2016; Grenham et al., 2011; Leigh et al., 2023; Peirce and Alviña, 2019; Tofani et al., 2025). This increased permeability enables the translocation of microbial-associated molecular patterns (MAMPs), such as LPS into the bloodstream (de Punder and Pruimboom, 2015; Miller and Raison, 2016), which in turn contributes to sustained systemic inflammation and promotes neuroinflammation in the central nervous system, further enhancing the HPA axis activity and the risk for the development of mental illnesses (Berthoud and Neuhuber, 2000; Beurel et al., 2020; Gola et al., 2013; Khandaker et al., 2014; Kivimaki et al., 2014; Lindqvist et al., 2014; Michopoulos et al., 2017; Miller and Raison, 2016; Peirce and Alviña, 2019; Reber et al., 2016a; Rook et al., 2012). These findings support the existence of a strong bidirectional connection between the gut microbiome, the immune as well as the neuroendocrine system, commonly known as microbiome-gut-brain axis (Carabotti et al., 2015; Dinan and Cryan, 2017; Grenham et al., 2011; Sherwin et al., 2016).

Studies in patients diagnosed with MDD demonstrated a reduction in microbial diversity and a significant shift in bacterial taxa. For instance, Hu et al. (2023) found a relationship between microbial diversity and MDD severity. The authors could determine specific bacterial taxa being affected depending on the severity of MDD.

Briefly, *Bacteroides* were found to be increased in moderate and severe MDD, while *Ruminococcus* and earlier designated *Eubacterium* were mainly diminished in severe MDD patients (Hu et al., 2023). In a large fecal microbiome population study in 2019, researchers were able to identify significant associations of the specific gut microbiome taxa, the quality of life and the status of depression (Valles-Colomer et al., 2019).

In detail, positive association was found between bacteria *Coprococcus* and *Dialister* and quality of life, while they were inversely related to depressive symptoms (Valles-Colomer et al., 2019). A study of Kelly et al. (2016) showed that transferring the gut microbiome of MDD patients to germ-free rats *via* fecal transplantation resulted in the development of depressive symptoms such as anhedonia, anxiety-related behavior and changes in the tryptophan metabolism in recipient rodents. These findings lead to the assumption of at least in part microbiota-driven symptomatology in MDD (Kelly et al., 2016).

Beyond depression, microbiome imbalance has also been reported in patients suffering from PTSD (Gao et al., 2022). In frontline healthcare workers exposed to stressful events during the COVID-19 pandemic, alpha (α-)diversity represented by the Chao1 index and observed amplicon sequence variants (ASVs), both indicating the richness of the gut microbiome, was found to be significantly reduced compared to second-line healthcare workers and persisted at least over half a year (Gao et al., 2022). Another clinical study reported significantly decreased levels of phyla such as Actinobacteria, Lentisphaerae and Verrucomicrobia in individuals affected by PTSD versus trauma-exposed individuals non-affected by PTSD (Hemmings et al., 2017), implicating a link between the microbial composition and the disease pathology.

As demonstrated in the aforementioned human studies, the gut microbiome has emerged as a critical factor in chronic inflammatory psychiatric disorders. However, changes in the microbiome of chronically stressed animals remain unclear, despite animal studies offering valuable opportunities to investigate how the microbiome interacts with stress-related neurocircuits. For instance, a preclinical study using the single prolonged stress model to induce PTSD-like symptoms in mice (Khan et al., 2025) reported increased intestinal permeability and significant gut dysbiosis in male mice of the PTSD group (Khan et al., 2025). In another PTSD-related preclinical study, stress was induced in males using a modified 10-day “cage-within-cage resident-intruder” model (Gautam et al., 2018). This study found no significant differences in α-diversity metrics, including Chao1, Simpson and Shannon indices, which estimate bacterial community richness and evenness within individual fecal samples

(McGuinness et al., 2022). In contrast, β-diversity analysis, which compares microbial communities between groups (McGuinness et al., 2022), revealed an increased Firmicutes/Bacteroidetes (F/B) ratio. This shift appeared on the first day in stressed mice and by the third day in controls, with an increase in *Firmicutes* and a decrease in *Bacteroidetes* (Gautam et al., 2018). This change reflects a deviation from the typical healthy proportions of Firmicutes (~ 31 %) and Bacteroidetes (~ 62 %) in mice (Gu et al., 2013).

Of note, both control and aggressor exposed mice were restricted from food and water during the 6 h experimental sessions which might have contributed to the observed microbiome alterations (Gautam et al., 2018).

Microbiome analysis in mice exposed to the CSC paradigm further supports the hypothesis that chronic psychosocial stress alters the gut microbiome (Reber et al., 2016b). CSC mice exhibited a marked increase in Proteobacteria, including Helicobacter spp., and Bacteroidetes, particularly *Paraprevotella*, which correlated positively with histological colon damage (Reber et al., 2016a; Reber et al., 2016b). Given that Helicobacter is known to induce colitis in immunoregulatory-deficient mice (Burich et al., 2001), a comparative study using two CSC setups, one with Helicobacter spp.-positive and the other with Helicobacter spp.-negative resident mice, demonstrated that colonization by resident-derived Helicobacter spp. contributes to CSC-induced colitis (Langgartner et al., 2017).

Chronic stress during gestation has also been shown to disrupt microbial homeostasis (Antonson et al., 2020; Gur et al., 2017; Jašarević et al., 2017; Zijlmans et al., 2015) and compromise intestinal barrier integrity in the mother (Kiecolt-Glaser et al., 2018), leading to an intrauterine inflammatory response (Spinillo et al., 2014; Turner et al., 2012) with long-term consequences for fetal immune development (Mor and Cardenas, 2010). For instance, Zijlmans et al. (2015) reported increased relative abundances of Proteobacteria, including *Escherichia*, *Serratia* and *Enterobacter*, and decreased relative abundance of Bifidobacteria and lactic acid-producing bacteria such as *Lactobacillus*, *Lactococcus* and *Aerococcus* in children of mothers who experienced high cumulative stress. These findings align with those of Galley et al. (2023), who observed positive associations between Bifidobacteria or lactic acid bacteria and antiinflammatory cytokine levels, respectively.

In a preclinical study by Sun et al. (2021), chronic variable stressor exposure from pregnancy day (PD) 10 until birth (Howerton et al., 2013) disrupted gut barrier function in weaned offspring. This was indicated by a significant reduction in the expression of tight junction proteins such as zonula occludens (ZO)-1, claudin-3, occludin and ZO-1 mRNA. These results were further supported by decreased claudin-3 protein levels, as measured by Western

blot, in PS offspring. Secretory immunoglobulin A (sIgA), as key component of immune defense, plays a crucial role in maintaining mucosal immunity in the small intestine. To assess potential intestinal immune impairment, sIgA-positive cells in the lamina propria were examined and found to be reduced in three-week-old pups exposed to PS. This finding led to the suggestion that besides compromising the mechanical barrier integrity, stress also impairs mucosal immune defense *in utero* (Sun et al., 2021).

In addition to the microbiome-gut-brain axis, there is another recognized interaction between the gut microbiome and skeletal system, referred to as microbiome-gut-bone axis (Zaiss et al., 2019). Chronic psychosocial stress has been associated with impaired bone metabolism contributing to conditions such as long-bone growth retardation, osteoporosis or an increased risk of bone fracture (Calarge et al., 2014; Gebara et al., 2014; Glaesmer et al., 2011; Glaesmer et al., 2012). Accordingly, both clinical and preclinical studies investigating bone metabolic disorders have also reported gut dysbiosis (José Neto et al., 2025; Liu et al., 2022a; Waldbaum et al., 2023). Schimmele et al. (2025) recently published that early life (postnatal) stress, induced *via* the established MS model, promotes femoral osteoclast activity in females, but not in males, which was linked to transient microbial alterations in α-diversity and F/B ratio. Moreover, long-lasting effects were observed in femoral tissues, including increased mRNA expression levels of proinflammatory cytokine *Il6*, and osteoclastic markers such as Cathepsin K and Receptor Activator of NF-κB Ligand (RANKL) (Schimmele et al., 2025). Based on these findings, and consistent with a previous study showing that IL-6 induces RANKL expression in osteoblasts, thereby activating osteoclastogenesis (McGregor et al., 2019), the authors hypothesized that postnatal stress-induced, sex-specific reduction in fecal F/B ratio contributes to sustained immune activation, ultimately leading to an osteopenic bone phenotype in female mice (Schimmele et al., 2025). Together, these insights underscore the critical role of the gut microbiome in meditating physiological effects of chronic psychosocial stress, with implications ranging from immune dysregulation to neurodevelopmental and skeletal outcomes.

As described above, chronic stress during pregnancy, i.e., prenatal stress, can affect the intestinal health of the offspring. The following section will specifically address the immunological and behavioral consequences of chronic PS from a clinical and preclinical perspective.

Prenatal stress as driver for long-term health risks

In accordance to the predictive proinflammatory cytokine theory in stress-induced psychiatric mood disorders, a long-term human study showed that adults whose mothers suffered from stress-induced depression during pregnancy had elevated plasma concentrations of CRP 25 years after birth, indicating peripheral inflammation (Plant et al., 2016). In support, PS induced by restraining during late pregnancy in female mice led to elevated hippocampal expression of proinflammatory *Il1b* and *Tnf* mRNA and activated microglial cells in the cornu ammonis region in adult male offspring (Diz-Chaves et al., 2013).

The latter was further aggravated following an acute immune challenge using LPS (Diz-Chaves et al., 2013). Growing preclinical evidence highlights sex-specific vulnerability to PS, with long-term consequences for stress-sensitive immunological and neurological systems (Bale, 2016; Howerton and Bale, 2012; Mueller and Bale, 2008; Suenaga et al., 2012). Although the placenta expresses the enzyme 11β-hydroxysteroid dehydrogenase type 2 (Benediktsson et al., 1997; Holmes et al., 2006), which converts active CORT into inactive metabolites (cortisone in humans, 11β-dehydrocorticosterone in rodents), and thereby limiting fetal exposure to only about one-fifth of maternal CORT (Gitau et al., 1998), it nevertheless appears to respond to maternal stress in a sex-specific manner (Bale, 2016; Bronson and Bale, 2014; Howerton and Bale, 2012; Mueller and Bale, 2008; Musillo et al., 2025; Seckl et al., 2000). Further, it contributes to different manifestations in male and female offspring in early childhood with significant impact on mental health in later life (Goldstein et al., 2021; Gur et al., 2019; Gur et al., 2017; Jašarević et al., 2017; Kalinichenko et al., 2023; Robinson et al., 2021).

Despite growing interest, rodent studies often report inconsistent findings regarding sex-specific behavioral outcomes of PS. For instance, research groups showed that prenatal paradigms affect predominantly emotional traits in males (Gur et al., 2019; Mueller and Bale, 2008), while others found increased anxiety- and depressive-related behavior predominantly in females, reflecting more the observations in human studies (Behan et al., 2011; Bowman et al., 2004; Gur et al., 2017). Moreover, there is evidence that the timing of stress exposure during gestation plays a critical role in determining sex-specific behavioral outcomes. While male animals are more affected by early PS exposure, females tend to exhibit more pronounced behavioral changes when stress occurs during late gestation (Li et al., 2008; Mueller and Bale, 2008). Similar patterns have also been observed in humans (de Bruijn et al., 2009). These behavioral alterations are associated with region- and sex-specific structural (Behan

et al., 2011) and inflammatory (Diz-Chaves et al., 2013) changes in the brain, underscoring the differential neurobiological vulnerability induced by prenatal stress.

Most murine studies investigating PS use restraining as primary stressor in pregnant dams (Antonson et al., 2020; Azuma et al., 2017; Barbazanges et al., 1996; Gur et al., 2017; Kalinichenko et al., 2023; Ma et al., 2024; Pascuan et al., 2014; Schmidt et al., 2017; Zhang et al., 2021). However, to more accurately model psychosocial stress, the inclusion of a social component is essential. In the following work, a validated rodent model was employed that combines daily restraint stress (1 h twice per day, 3 h apart) with the social instability paradigm (Hillerer et al., 2011) lasting from PD 4 to PD 16.

Unlike PS models that rely solely on restraining pregnant females, the addition of a psychosocial stressor, like in the SIP, has been shown to elicit a stronger CORT response than restraining alone (Koolhaas et al., 2011). Notably, the PS model employed in the present PhD thesis has not yet been used to examine sex-specific behavioral, physiological, and immunological outcomes in offspring. Therefore, the results of a 10-day PS model with variable stressors (twice per day, 2 h each; from PD 6.5 to PD 16.5) and a psychosocial stressor such as overnight cage mate change (Zoubovsky et al., 2020) may provide an indication of the effects on male and female offspring (Zoubovsky et al., 2022).

Zoubovsky et al. (2022) reported behavioral, physiological, and neurodevelopmental alterations following psychosocial PS exposure. PS offspring showed an anxiety-like phenotype demonstrated by significantly less time spent in the light zone during the light-dark box test, while no changes in locomotion were observed in the OF test. Sociability and associative learning were not affected by PS (Zoubovsky et al., 2022). However, depressive-like behavior was observed, as PS offspring showed a significant reduction in time spent immobile during the forced swim test (FST) compared to respective controls (Zoubovsky et al., 2022). The authors interpreted this finding as reflecting a more active coping strategy in response to acute stress (i.e., swimming). This suggests altered stress responsiveness that may indicate increased vulnerability to emotional disturbances such as anxiety (Zoubovsky et al., 2022), which is linked to *in utero* stress (Mueller and Bale, 2008). Moreover, to assess anhedonia, a core symptom of depression characterized by a reduced ability to experience pleasure (Moreau, 2002), the sucrose (or saccharin) preference test (SPT) revealed a significant reduction in preference for 4 %-sucrose solution in PS offspring versus controls (Zoubovsky et al., 2022). In this study, statistical analysis did not reveal a significant sex-PS interaction indicating that male and female PS offspring were similarly affected at least in the behavioral parameters (Zoubovsky et al., 2022).

Furthermore, neuroendocrine abnormalities were observed in PS females exhibiting elevated serum CORT levels after FST exposure (Zoubovsky et al., 2022). At the circadian peak, the expected physiological sex differences in HPA axis activity were present, with females showing higher CORT concentrations (Rao and Androulakis, 2017). PS also induced complex and sex-specific gene expression changes in the amygdala, suggesting dysfunctional neurotransmitter systems. In males, PS led to the downregulation of genes involved in synaptic transmission and altered mRNA transcripts encoding synaptic proteins involved in glutamatergic and GABAergic neurotransmission (Zoubovsky et al., 2022). In contrast, PS in females resulted in the upregulation of genes associated with increased noradrenergic activity and increased anxiety-related behaviors (Zoubovsky et al., 2022).

Given that early childhood seems to be a critical period for the development of chronic stress- and inflammation-associated diseases, David P. Strachan (1989) proposed that a lower microbial load during this stage of life might contribute to the rise in atopic allergies and autoimmune diseases in industrialized nations. His observations contributed to the foundation of the later "hygiene hypothesis", which posits that reduced exposure to diverse environmental microorganisms disrupts the healthy development of the immune system, thereby increasing the risk for chronic inflammatory diseases (Pfefferle et al., 2021; Strachan, 2000).

"Old Friends" hypothesis

In support of Strachan's "hygiene hypothesis", several studies of immigrants moving from low- to highly-industrialized countries or from traditional rural to urban areas suggest that early and/or prolonged microbial exposure can offer protection against inflammatory disorders later in life (Ahlgren et al., 2012; Eldeirawi et al., 2009; Hou et al., 2009; Li et al., 2011; Pereg et al., 2008; Riedler et al., 2001; Rottem et al., 2005; Söderström et al., 2012). For instance, researchers have identified that, despite rising global incidence, first-generation immigrants to Canada had lower IBD rates than native Canadians, though their Canadian-born children showed increased risk (Benchimol et al., 2015). Notably, children of mothers from East Asia and the Pacific, Eastern Europe, Central Asia, Latin America and the Caribbean exhibited lower incidence of IBD compared to children of non-immigrant parents, suggesting that certain protective environmental factors might persist across generations. In contrast, children with maternal origin from the Middle East, Western Europe and North America showed IBD incidence rates comparable to those of Canadian families. The authors also highlighted that early life exposure to Canadian environment increases the risk of IBD, potentially due to a combination of factors including dietary changes, increased antibiotic use,

shifts in hygiene standards or variations in birth methods (e.g., sterile Caesarean sections), all of which might significantly alter the (gut) microbiome in early childhood (Benchimol et al., 2015).

Similar results were found in immigration studies investigating the risk of type 1 diabetes in Sweden (Hussen et al., 2013a; Hussen et al., 2013b; Söderström et al., 2012), the prevalence of multiple sclerosis in Norway (Berg-Hansen et al., 2015), and the susceptibility to asthma in Ethiopian immigrants in Israel (Rosenberg et al., 1999) or Mexican Americans in the United States (Eldeirawi et al., 2009) as well as other allergies (Cabieses et al., 2014). In line with the aforementioned, immigration studies considering psychiatric disorders such as depression (Breslau et al., 2011), anxiety and mood disorders (Breslau et al., 2009), autism (Keen et al., 2010) or schizophrenia (Dealberto, 2010) reported an increased prevalence in urban or westernized environments indicating that a reduction in microbial diversity compared to rural environments with a wide range of microbial exposure might play a pivotal role.

Moreover, a human study investigating the immune response following acute psychosocial stress exposure using the TSST (Kirschbaum et al., 1993) in healthy male volunteers raised in urban areas in absence of pets relative to individuals raised in rural areas in the presence of farm animals showed a pronounced increase in the count of PBMCs and in a prolonged inflammatory response due to increased plasma IL-6 concentrations (Böbel et al., 2018; Langgartner et al., 2020). Extending these findings, Langgartner et al. (2025) studied healthy urban-raised men with or without childhood pet contact and found that, following the TSST, those without pets showed greater neutrophil mobilization and elevated plasma IL-6 levels. Additionally, the authors were able to show that the lack of contact to pets during childhood in an urban environment results in long-lasting intestinal barrier dysfunction, as confirmed by increased plasma levels of LPS-binding protein (LBP) at the time point prior to the TSST (Langgartner et al., 2025). LBP produced in the liver and responsible for the presentation of LPS to pattern recognition receptors (PRRs) on the surface of various immune cells is a reliable long-term marker for bacterial endotoxin exposure (Voigt et al., 2022). However, the findings of an impaired intestinal barrier did not contribute to differences in the composition of the host's microbiome and respective metabolites, as salivary microbial α- and β-diversity were comparable between both cohorts (Langgartner et al., 2025).

Therefore, considering all the insights of the abovementioned studies, it seems that more natural and microbial-enriched environments resulting in an increased diversity of the human (gut) microbiome seem to support physical and mental health (Clemente et al., 2015; McBurney et al., 2019; Rook, 2013; Spragge et al., 2023; Walker et al., 2021).

These findings provide compelling evidence for the so-called "Old Friends" hypothesis which posits that reduced exposure to evolutionary co-adapted microorganisms might be responsible for immunoregulatory function (Langgartner et al., 2019; Raison et al., 2010; Rook, 2013; Rook et al., 2014). Microorganisms referred to as "Old Friends" were categorized in 1) commensal microbiota, residing in the gut, airway, skin, genitourinary tract and the oropharyngeal region, all of which play essential roles in physiological processes (Hanski et al., 2012; Hoffmann et al., 2013; Naik et al., 2012; Round et al., 2011), 2) environmental microbiota from sources such as water, air and soil with which individuals are in regular contact (Rook et al., 2013a; Rook et al., 2014), and 3) microorganisms from our evolutionary past, like saprophytic mycobacteria (Amoroso et al., 2021), helminths (Babu et al., 2006), *Helicobacter pylori* or the hepatitis A virus (Wolfe et al., 2007) that historically caused subclinical infections.

Many of these microbes are now largely absent from modern environments and are therefore termed "old" infections (Rook et al., 2014). The latter have developed sophisticated strategies to avoid provoking harmful inflammation by inducing immunoregulatory responses, allowing them to coexist with the host without causing damage (Rook, 2013). For instance, helminthic parasites promote immunoregulatory pathways, as their survival depends on maintaining the host's physiological stability (Grainger et al., 2010). Grainger et al. (2010) demonstrated that biologically active *Heligmosomoides* (*H.*) *polygyrus* excretory-secretory antigen drives the expression of the transcription factor FoxP3 in $CD4^+CD25^+$ Tregs *ex vivo*. Moreover, in a murine model of ovalbumin-induced IBD, infection with *H. polygyrus* resulted in a marked increase of IL-10 concentrations which abrogated the intestinal inflammation (Leung et al., 2012).

In addition to directly restoring immunoregulation by inducing antiinflammatory cytokine-producing Tregs, an indirect mechanistic approach of the "Old Friends" hypothesis might be the promotion of beneficial bacterial taxa such as species of the families Lactobacillaceae (Walk et al., 2010) or Lachnospiraceae, Ruminococcaceae and Bifidobacteriaceae which are known to produce short-chain fatty acids (SCFAs), such as butyrate (Depner et al., 2020; Eeckhaut et al., 2013; Hamer et al., 2008; Lee et al., 2020; Louis and Flint, 2009; Marteau, 2013; Nogal et al., 2021).

Butyrate has been shown to counteract histone H3 deacetylase-mediated inhibition of the FoxP3 gene, thereby contributing to the differentiation of Tregs (Arpaia et al., 2013). In line with this, Gao et al. (2022) reported that the relative abundance of several butyrate-producing species including members of the Lachnospiraceae family, such as *Roseburia*, were reduced in healthcare workers suffering from PTSD. Similarly, Valles-Colomer et al. (2019) identified *Roseburia* as a potential serotonin producer associated with positive outcomes in antidepressant treatment. These findings support the concept that targeting immunoregulatory pathways through the "Old Friends" approach might offer a promising therapeutic strategy for stress-related psychiatric disorders.

The immunoregulatory nature of *Mycobacterium vaccae*

Taking advantage of the immunoregulatory properties attributed to the "Old Friends", various studies investigating the negative consequences of stress have employed rapidly growing mycobacteria (RGM) (Amoroso et al., 2021; Rook et al., 2004), particularly abundant saprophytic, heat-inactivated, non-pathogenic strains of *M. vaccae*. Among these, the *M. vaccae* NCTC 11659 has been widely studied as a potential therapeutic agent (Amoroso et al., 2020a; Amoroso et al., 2019; Desmond et al., 2023; Fonken et al., 2018a; Foxx et al., 2020; Frank et al., 2018a; Gebauer et al., 2025; Hassell et al., 2019; Langgartner et al., 2023a; Loupy et al., 2019; Loupy et al., 2021; Lowry et al., 2007; Reber et al., 2016b; Schimmele et al., 2025; Siebler et al., 2018; Smith et al., 2019).

M. vaccae NCTC 11659, also designated as CECT (Colección Española de Cultivos Tipo) 96464^T or DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) 107316^T, was reclassified in 2018 based on 16S rRNA gene and genome sequence data (Nouioui et al., 2018). It is now validated as *M. kyogaense sp. nov.*(Nouioui et al., 2018), named after Lake Kyoga in Uganda, where it was originally isolated from mud by Stanford and Paul (1973). In line with previous studies that refer to the strain as *M. vaccae* NCTC 11659, I will retain this designation throughout the thesis in order to avoid confusions with other *M. vaccae* strains. Detailed information on *M. vaccae* NCTC 11659 is provided in the review by Amoroso et al. (2021).

Briefly, evidence for the exceptional immunoregulatory properties of widely used *M. vaccae* NCTC 11659 was shown in both preclinical and clinical studies (Stanford et al., 2009), particularly *via* its special assembly of the dual-membrane structure including an outer waxy membrane rich in mycolic acids and free lipids, a polysaccharide cell wall, as well as an inner cytosolic membrane (Batt et al., 2020; Dulberger et al., 2020; Ishikawa et al., 2017).

Macrophages and dendritic cells (DCs) can recognize molecules in the outer membrane through PRRs such as TLRs, nucleotide-binding oligomerization domain-like receptors, and C-type lectin receptors (Ishikawa et al., 2017). This recognition triggers DC maturation, followed by immune response modulation. In the case of *M. vaccae* NCTC 11659, naïve T cells differentiate into T helper (Th) 1 cells (Fowler et al., 2012; Stanford and Stanford, 2012) and Tregs (Reber et al., 2016b; Stanford et al., 2009; Zuany-Amorim et al., 2002).

However, *M. vaccae* NCTC 11659 appears to influence a range of cellular and molecular immunoregulatory mechanisms. One proposed mechanism is the ability to promote Th1 polarization, while shifting the immune response away from a Th2-dominated profile. This effect has been observed in studies of Th2-associated conditions such as lepromatous leprosy (Abbot et al., 2002; Yamamura et al., 1992), tuberculosis (Stanford et al., 2004), and asthma (Wang and Rook, 1998). Specifically, *M. vaccae* NCTC 11659 facilitates the maturation of DCs (Le Bert et al., 2011) and myeloid CD $11b^+$ cells (Bazzi et al., 2015), and increases the number of γδ T cells with subsequent induction of Th1 cytokine secretion such as IFNγ and TNFα (Fowler et al., 2012).

Notably, repeated s.c. administrations of *M. vaccae* NCTC 11659 induces the maturation of $CD4^+CD25^+$ Tregs, as evidenced by experiments involving the depletion of $CD25^+$ Tregs using an antibody prior to exposure to a psychosocial stressor (Reber et al., 2016b). This induction is accompanied by increased secretion of the antiinflammatory cytokines IL-10 and TGFβ (Reber et al., 2016b). These findings were previously supported by a study using a murine model for airway inflammation (Zuany-Amorim et al., 2002). Furthermore, Smith et al. (2019) demonstrated in mice that *M. vaccae* NCTC 11659 synthesizes lipid 10(*Z*)-hexadecenoic acid, which binds to peroxisome proliferator-activated receptor alpha (PPARα) and subsequently downregulates genes within the NF-κB signaling pathway. This interaction suppresses LPS-induced inflammation in peritoneal macrophages (Smith et al., 2019). In addition, *M. vaccae* NCTC 11659 modulated stress-induced neuroinflammation in rats by promoting an antiinflammatory phenotype in hippocampal microglia (Fonken et al., 2018a; Fonken et al., 2018b; Frank et al., 2018a; Frank et al., 2018b), ameliorated age-related microglia activation in the hippocampus and amygdala (Sanchez et al., 2022), and prevented negative effects of sleep deprivation (Bowers et al., 2020).

To investigate resilience-enhancing effects of *M. vaccae* NCTC 11659 on negative outcomes of chronic psychosocial stress such as general and social anxiety-related behaviors, systemic inflammation, and both spontaneous and DSS-induced colitis, Reber et al. (2016b) administered the RGM s.c. (0.1 mg in 0.1 mL borate-buffered saline (BBS)) on days -21, -14,

and -7 before exposure to CSC. Preventive immunization with *M. vaccae* NCTC 11659 in CSC-exposed mice resulted in the following beneficial effects: 1) stabilization of the gut microbiome, 2) increased percentage of Tregs in mesLNCs, 3) prevention of CSC-induced colitis and suppression of proinflammatory cytokine secretion from *ex vivo* anti-CD3-stimulated mesLNCs, 4) prevention of exacerbation of DSS-induced colitis, 5) reduction of anxiety-like behavior, and 6) an increased number of $CD4^+CD25^+FoxP3^+CD62L^-$ mesLNCs, enhanced Treg populations when compared to BBS-treated CSC-exposed controls (Reber et al., 2016b). Of note, the impact of CSC exposure on Treg functionality has not yet been investigated. Additionally, repeated s.c. administration of *M. vaccae* NCTC 11659 prior to CSC exposure in own-bred male animals conferred partial protection against CSC-induced alterations in bone morphology (Gebauer et al., 2025).

From a translational perspective, predicting psychosocial stressor exposure in humans is challenging. To address this, Amoroso et al. (2020a) investigated whether repeated s.c. administration of *M. vaccae* NCTC 11659 during CSC exposure, specifically on days 2, 8, and 15, would provide similar stress-protective effects as the preventive approach. Their findings demonstrated that *M. vaccae* NCTC 11659, when administered during ongoing stress exposure, effectively prevented CSC-induced general and social anxiety-like behaviors, comparable to the preventive strategy (Amoroso et al., 2020a).

Furthermore, Mazzari et al. (2023) reported that in adult female mice repeated s.c. injections of *M. vaccae* NCTC 11659 prior to SIP exposure only prevented the increase in splenic neutrophil counts and the elevation of the antiinflammatory cytokine IL-4 levels. Of particular importance to the present thesis, Schimmele et al. (2025) recently showed that repeated s.c. administration of *M. vaccae* NCTC 11659 following postnatal ELA, i.e., MS (3h per day, over 14 consecutive days after birth), protected the female offspring against the development of a moderate osteopenic phenotype. This protective effect was associated with transient alterations in gut microbiome composition and prolonged activation of the HPA axis and the immune system. In contrast, male offspring did not exhibit bone-related effects following MS, suggesting a sex- and developmental stage-specific vulnerability of murine bone to early life stress (Schimmele et al., 2025).

As most preclinical studies (Fonken et al., 2018a; Frank et al., 2018a; Lowry et al., 2007; Reber et al., 2016b; Zuany-Amorim et al., 2002) have used the s.c. route to administer *M. vaccae* NCTC 11659, and also intradermal (i.d.) administration is also employed in human studies (Abbot et al., 2002; Camporota et al., 2003; Cananzi et al., 2013; Johnson et al., 2000; Lehrer et al., 1998), alternative less invasive administration routes have been explored.

These routes include intranasal (i.n.; 0.1 mg per mouse per administration) or i.g. (0.1 mg per mouse per administration) delivery, investigated by Amoroso et al. (2019) and Langgartner et al. (2023a), respectively, in the context of chronic psychosocial stress in adult C57BL/6N male mice. Although reduced bioavailability of *M. vaccae* NCTC 11659 *via* mucosal routes might be expected, due to a shorter systemic absorption phase compared to s.c. injection, both studies provided evidence that significant stress-protective effects can still be achieved through i.n. (Amoroso et al., 2019) and i.g. (Langgartner et al., 2023a) administration.

In order to determine whether these stress-protective immunoregulatory properties of *M. vaccae* NCTC 11659 are exclusive to RGMs, Langgartner et al. (2024) conducted a study using the environmental saprophytic non-tuberculous mycobacterium (NTM) *M. aurum* DSM 33539. Repeated i.g. administration of heat-inactivated *M. aurum* DSM 33539 prior to CSC exposure stabilized gut microbiome composition in adult C57BL/6N male mice. This is notable because CSC exposure increased the relative abundance of colitogenic phyla such as Desulfobacterota and Deferribacterota (Langgartner et al., 2024). Furthermore, *M. aurum* DSM 33539 prevented stress-induced aggravation of DSS-induced colitis, an effect previously observed with *M. vaccae* NCTC 11659 (Langgartner et al., 2024). These findings suggest that the protective effects of RGMs as "Old Friends" in mitigating the negative consequences of chronic psychosocial stress might be generalizable to other NTMs as well.

To further explore the stress-protective and immunoregulatory potential of mycobacteria as preventive therapeutic agents, the closely related strain *M. vaccae* ATCC 15483^T was selected for additional investigation. This strain, also referred to as NCTC 10916^T (UK Health Security Agency, 1973) or DSM 43292^T (Nouioui et al., 2018) was originally isolated from cow dung in Austria by Boenickse and Juhasz (1964) and later obtained from cow milk for laboratory cultivation (Rodríguez-Güell et al., 2006). Unlike the NCTC 11659-strain, which forms only rough colonies, the ATCC 15483^T-strain exhibits both rough and smooth colonies on solid culture media, depending on the incubation temperature (Rodríguez-Güell et al., 2006). These two *M. vaccae* ATCC 15483^T colony phenotypes differ in biochemical composition, surface structure, and immunogenicity (Rodríguez-Güell et al., 2006). Rodríguez-Güell et al. (2006) demonstrated that the rough variant induces a Th1-biased immune response, characterized by increased production of IFNγ and IL-12, when administered s.c. in mice. In contrast, the smooth morphotype elicits a less pronounced Th1 response, but induces higher concentrations of the antiinflammatory IL-10 (Rodríguez-Güell et al., 2006). Further lipid profiling revealed the presence of an extracellular long-chain saturated

polyester in the smooth, but not the rough, colony type. This compound was termed RC ("red color") due to the appearance of red stain in combination with anthrone (Rodríguez-Güell et al., 2006).

To evaluate the stress-protective effects of *M. vaccae* ATCC 15483^T and compare them with those of *M. vaccae* NCTC 11659, Loupy et al. (2021) employed the inescapable tail shock (IS) paradigm, as previously described by Frank et al. (2018a). Anxiety-related behaviors were assessed both before and after the stressor exposure using the juvenile social exploration test (Christianson et al., 2008).

In this paradigm, an experimental adult male rat was confronted with a juvenile male rat (28-32 days old), and exploratory behaviors such as sniffing, pinning, licking and allogrooming were quantified (Frank et al., 2018a). Exposure to IS induced anxiety-like defensive behaviors, which were prevented by pre-immunization with *M. vaccae* ATCC 15483^T (Loupy et al., 2021). These behavioral effects were accompanied by changes in metabolic-immune signaling. Specifically, the IS-induced decrease in hepatic *Pparg* mRNA expression and increase in hippocampal *Il6* mRNA expression were both prevented by *M. vaccae* ATCC 15483^T. These insights indicate that the stress resilience effects of environmental saprophytic mycobacteria are not exclusive to a single representative strain, but may extend across multiple representatives (Loupy et al., 2021).

In a recently published preclinical study conducted by Desmond et al. (2024), eleven weekly repeated s.c. administrations with *M. vaccae* ATCC 15483^T effectively prevented negative sequelae associated with "Western"-style diet, characterized by high fat, high sugar and low fiber content. The performance of the study was motivated by rising prevalence of non-communicable inflammatory diseases, including obesity, in modern urban societies (Welsh et al., 2024). In the experimental model, obesity was mimicked by excessive weight gain, accumulation of visceral adipose tissue, and elevated concentrations of plasma leptin (Desmond et al., 2024). Although *M. vaccae* ATCC 15483^T did not significantly alter the α- or β-diversity of the gut microbiome in experimental diet-exposed mice, the authors suggested that it might exert its effects through modulation of metabolic-immune signaling downstream of the microbiome (Desmond et al., 2024), potentially by directly targeting the innate and adaptive immune pathways (Amoroso et al., 2021). Notably, immunization with *M. vaccae* ATCC 15483^T reduced baseline levels of neuroinflammatory markers, including *Nfkbia* and *Nlrp3* mRNA expression as well as microglial priming in the hippocampus, in addition to decreased anxiety-like defensive behaviors assessed by the EPM (Desmond et al., 2024).

Importantly, in the context of chronic psychosocial stress, particularly in the prenatal phase, *M. vaccae* ATCC 15483^T has not been investigated for its potential to prevent stress-induced negative consequences across generations so far.

Aims of this thesis

Chronic psychosocial stress is a risk factor for the development of numerous somatic and psychiatric disorders. Exposure to chronic stress can occur across multiple stages in life, with ELAs influencing stress vulnerability in later life. Although the underlying mechanisms remain largely unknown, stress-related disorders are associated with a dysregulated immune response and chronic low-grade inflammation, and an impaired Treg compartment playing a central role. Epidemiological data suggest that, in addition to pronounced sex differences, growing up in urban rather than rural environments influences the prevalence of stress-related disorders. These urban-rural differences could be linked to the extent of contact with immunoregulatory microbes. Supporting the theory that stress-related disorders are accompanied by deficits in immunoregulation, reduced exposure to immunoregulatory microorganisms ("Old Friends"), particularly during early pre- and postnatal life, might contribute to a stress-induced increase of immune (re-)activity and inflammation in modern industrialized societies. Consistent with this hypothesis, a growing body of evidence indicates that "Old Friends", such as non-pathogenic immunoregulatory RGMs, including *M. vaccae* NCTC 11659 and *M. vaccae* ATCC 15483^T, exert immunoregulatory and stress-protective effects when administered repeatedly prior to or during chronic stressor exposure and, therefore appear to be a novel and innovative preventive or treatment strategy.

In this context, with the present thesis, I investigate whether i.g. administration of a heat-killed preparation, non-pathogenic saprophytic RGM to nulliparous C57BL/6N female mice prior to mating can prevent CAS- or PS-induced behavioral, physiological and immunological negative consequences in male and female offspring. In previous studies on chronic psychosocial stress, *M. vaccae* NCTC 11659 was used as preventive and therapeutic immunoregulatory agent. With the recent work, I extend the immunoregulatory potential of another candidate, which is *M. vaccae* ATCC 15483^T, to an intergenerational preventive strategy against chronic psychosocial stress experienced in prenatal life or adulthood. It can be referred to as an intergenerational approach, as according to Klengel et al. (2016), an intergenerational transfer of effects occurs when the unborn F1 generation is directly exposed after conception or when adult germ cells of the F0 generation are exposed prior to conception.

Therefore, the aims in my thesis explore the following research questions:

Does repeated i.g. administration of *M. vaccae* ATCC 15483^{T} to female mice prior to mating intergenerationally protect their male and female offspring against the negative consequences of either

1) chronic adult stress (i.e., CSC in males, SIP in females) or

2) prenatal stress (i.e., alternating between restraining and SIP),

despite the experimental offspring has never been exposed directly to the RGM itself?

Materials and Methods

Materials

Animals

To obtain in-house bred experimental male and female C57BL/6N mice, nulliparous C57BL/6N females (15 – 20 g) and C57BL/6N breeder males (21 – 27 g, used for two rounds of mating) were purchased from Charles River Laboratories (Sulzfeld, Germany). Parental animals of both sexes were approximately 9 weeks of age. CD-1 male mice (30 – 35 g, purchased from Charles River Laboratories) served as the dominant, aggressive stressor in the CSC paradigm for adult male offspring. All specific pathogen-free mice were kept under standardized housing conditions, including a 12 h light-dark cycle (lights on at 0600 h during wintertime, and 0700 h during summertime), at 22 °C and 60 % humidity, in standardized polycarbonate mouse cages (22 cm length x 16 cm width x 14 cm height) with ad libitum access to tap water and standard mouse diet, unless stated otherwise below.

Table 1: Number of animals mated to obtain experimental mice

Mating	**nulliparous C57BL/6N females**		**breeding C57BL/6N males**
round 1 (Experiments 1, 3 and 4)	BBS *N* = 24	MvacATCC *N* = 24	24
round 2 (Experiment 1, 3 and 4)	BBS *N* = 8	MvacATCC *N* = 20	14
Mating	**nulliparous C57BL/6N females**		**breeding C57BL/6N males**
Experiments 2 and 5	**BBS** *N* = 22	**MvacATCC** *N* = 26	24

Buffers and Solutions

Table 2: List of buffers and solutions

Buffer	**Ingredients**
ACK lysis buffer (for splenocyte and PMN-MDSC- enriched PBMCs)	155 mM NH_4Cl, 10 mM $KHCO_3$, 10 nM EDTA
BBS	106 mM NaCl, 9.5 mM $Na_2B_4O_7*10H_2O$, 85 mM H_3BO_3, 1 % Tween 80, pH = 8.067, sterile filtered
FACS buffer	1x PBS, 10 % FCS, 0.1 % NaN_3
MACS buffer	1x PBS, 0.5 % BSA, 2mM EDTA
PBS (10x)	137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4
RPMI supplemented	10 % FCS, 50 U/ mL penicillin, 50 µg/ mL streptomycin
TBS (10x)	500 mM Tris, 1.5 M Nacl, pH 7.5
TBS-T	1x TBS, 0.1 % Tween

Table 3: List of media and kits

Medium or Kit	**Company**
16S rRNA gene amplicon sequencing and bioinformatics analysis	Novogene GmbH
AffinityScript qPCR cDNA Synthesis Kit	Agilent Technologies
Ampuwa H_2O	Fresenius
BCECF	Abcam
β-Mercaptoethanol	Gibco life Technologies
Bicinchoninic Acid Protein Assay Kit	Thermo Fisher Scientific
Brilliant III Ultra-Fast SYBR Green QPCR Master Mix	Agilent Technologies
BSA	BioFroxx
$CD4^+CD25^+$ Regulatory T Cell Isolation Kit	Miltenyi Biotech Incorporate
Cell extraction buffer	Invitrogen
CellTiter 96® Aqueous One Solution Assay	Promega
CellTrace™ CFSE Cell Proliferation Kit for flow cytometry	Invitrogen
Clarity™ Western ECL Substrate	Bio-Rad Laboratories
Complete (MINI) protease inhibitor	Roche Diagnostics GmbH
Corticosterone (CORT)	Sigma-Aldrich
Dextran	Sigma-Aldrich
DMEM	Gibco life Technologies
DMSO	Carl Roth
dPBS	Gibco life Technologies
FCS	Gibco life Technologies
Ficoll	GE Healthcare
FMLP	Sigma-Aldrich
FoxP3 Transcription Factor Staining Buffer Set Kit	eBioscience
HBSS	Sigma-Aldrich
HBSS	Gibco life Technologies, 0.1 % BSA
LPS	Sigma-Aldrich
Mouse LBP ELISA Kit	HycultBiotech
Penicillin-Streptomycin	Invitrogen
Permeabilization buffer	eBioscience
Phusion® High-Fidelity PCR Master Mix	New England Biolabs
Plasma Corticosterone ELISA Kit	IBL International GmbH
PowerSoil DNA extraction Kit	MoBio Laboratories
Protease Inhibitory Cocktail	Sigma-Aldrich
Qiagen Gel Extraction Kit	Qiagen
Qubit RNA CR Assay Kit	Thermo Fisher Scientific

Re-Blot Plus Strong Antibody Stripping Solution	Millipore GmbH
RNeasy Mini Kit	Qiagen
RPMI-1640	Sigma-Aldrich
Tissue Extraction Reagent I	Invitrogen
TruSeq® DNA PCR-Free Sample Preparation Kit	Illumina

Antibodies

Table 4: List of antibodies used for western blot analysis

Antibody	**Dilution**	**Company**
HRP-conjugated anti-mouse IgG antibody	1:5000	Cell Signaling Technology
HRP-conjugated chicken anti-goat secondary antibody	1:1000	R&D Systems
HRP-conjugated goat anti-mouse secondary antibody	1:10000	Jackson Immuno Research
Primary anti-mouse TLR4 antibody	1:1000	Santa Cruz Biotechnology
Primary goat anti-TLR2 antibody	1:200	R&D Systems

Table 5: List of antibodies used for flow cytometry

Marker	**Conjugate**	**Dilution**	**Company**
CD11b	APC-eFluor 780	1:100	BD Bioscience
Ly6C	APC	1:200	BD Bioscience
Ly6G	V450	1:400	BD Bioscience
CD4	APC-eFluor 780	1:400	Thermo Fisher Scientific
CD25	PE	1:200	Thermo Fisher Scientific
FoxP3	APC	1:100	Thermo Fisher Scientific

Table 6: List of antibodies used for stimulation

Antibody	**Company**
Anti-CD3 antibody	Thermo Fisher Scientific
Anti- CD28 antibody	Thermo Fisher Scientific

Primers

Table 7: List of primers used in PCR

Primers	**Company**
Forward: 5′-CTGAAGCTTTTGGCTTTGAG-3′	MWG Biotech

Reverse: 5′-CCACTGCCAAATTCTTTGG-3′	MWG Biotech
Complement C3 primers	Qiagen
HPRT1 primers (self-designed)	Biomers

Molecular biology materials

Table 8: List of molecular biology materials

Material	**Company**
4-20 % Mini-PROTEAN TGX Stain-Free Gel	Bio-Rad Laboratories
20-gauge gavage needle	FST
30 µm cell strainer	Miltenyi Biotech
70 µm cell strainer	Corning
96-well chemotaxis mini-chamber	Neuroprobe
96-well plate, flat-bottom	Falcon®, Corning Life Sciences
96-well plate, round-bottom	Falcon®, Corning Life Sciences
Centrifugation tube (15 mL, 50 mL)	Falcon®, Corning Life Sciences
EDTA-coated tubes	Sarsteadt
Heat-inactivated *Mycobacterium vaccae* ATCC 15483^{T}	Institute of Medical Microbiology and Hygiene, Ulm University Medical Center
KAPA2G Fast Hot Start Genotyping Mix	Thermo Fisher Scientific
LD separation columns	Miltenyi Biotech
MS separation columns	Miltenyi Biotech
Nitrocellulose blotting membranes	GE Healthcare
OctoMACS^{TM} Separator	Miltenyi Biotech
PageRuler Plus Prestained Protein Ladder	Thermo Fisher Scientific
Polycarbonate membrane, 3 µm porosity	Neuroprobe
QuadroMACS^{TM} Separator	Miltenyi Biotech
Syringes (2mL, 5 mL, single-use)	B. Braun Melsungen

Laboratory equipment

Table 9: List of laboratory equipment

Equipment	**Company**
BB15 CO_2 incubator	Thermo Fisher Scientific
ChemiDoc^{TM} XRS+ system	Bio-Rad Laboratories
Cubis MSE225P-000-DU Semi-Micro Balance	Sartorius Weighing Technology GmbH
Digital precision caliper	Mitutoyo
ELISA plate reader Fluostar Optima	BMG Labtech GmbH
Heraeus Megafuge 40R centrifuge	Thermo Fisher Scientific
Homogenizer	SpeedMill
InnuSPEED Lysis Tube P	Innuscreen GmbH

LSRII flow cytometer	BD Bioscience
Molecular Imager® ChemiDoc™ MP system	Bio-Rad Laboratories
Qiashredder™	Qiagen
qPCR cycler Mx3000P	Agilent Technologies
QuadroMACS™ Separator	Miltenyi Biotech
Qubit 2.0 fluorometer	Thermo Fisher Scientific
Skyscan 1172 scanning system	Bruker
ViCell CR cell counter	Beckman Coulter

Software

Table 10: List of software

Software	**Company**
Ascent	Version 2.6
BioRender	2022 BioRender
Ethovision™ XT	Version 11.5.1022, Noldus Information Technology
FlowJo™	Version 10, Tree Star
GIMP 2.6	Version 2.6.12
GraphPad Prism	Version 10.4.1.
Image Lab™ Software	Bio-Rad Laboratories
Microbial bioinformatics analysis	Novogene GmbH
Microsoft Office	Microsoft Corporation
Optima	BMG Labtech
Platform for cluster generation and sequencing by synthesis	Illumina
R/ RStudio 4.5.1	Version 4.5.1 Posit
Skyscan software (NRecon, DataViewer, CTAn, CTVox)	Bruker

Methods

1. Animals

Experimental male and female offspring were generated from nulliparous C57BL/6N female mice and the respective mating males as described in the "*Animals*" section of the Materials part and in the section "*Mating, birth and weaning procedure*" (see below). All experimental protocols were approved by the Committee on Animal Health and Care of the local government (Regierungspräsidium Tübingen, Germany) and were conducted in accordance with national and international guidelines on the ethical use of animals (ARRIVE guidelines 2.0 and EU Directive 2010/63/EU for animal experiments, Percie du Sert et al. (2020)). Every effort was made to reduce the number animals used to the minimum necessary and to minimize their suffering.

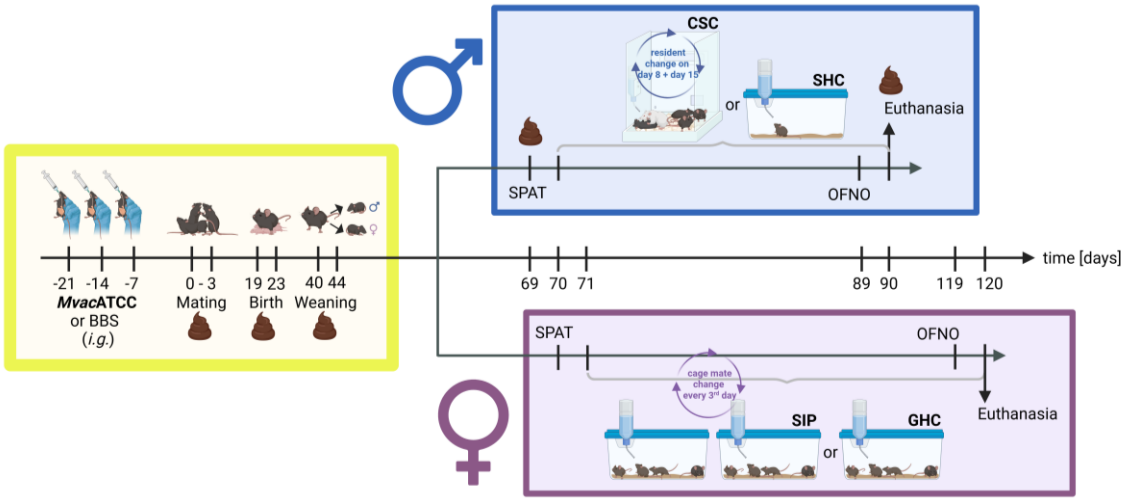
2. *Experimental procedures*

To address **aim 1** (**Timeline of Experiment 1**), either *M. vaccae* ATCC 15483^{T} (MvacATCC) or its vehicle Borate-Buffered Saline (BBS) was repeatedly administered i.g. to nulliparous female mice on days -21, -14, and -7 before mating (day 0-3), while they were housed in groups of four (Schiele et al., 2026). Pregnant females were left undisturbed throughout gestation in order to obtain experimental male and female offspring. Prior to birth (days 19-23), pregnant females were housed individually. Subsequently, maternal "on nest" behavior was observed during the first week after birth, excluding postnatal day 3, as the pup retrieval test was performed on that day. The litters together with their dams were kept in their home cages until day of weaning and sex determination (days 40-44). In the following, male and female offspring were housed in groups up to five siblings per cage. In order to avoid hierarchy establishment, male offspring were housed individually, while females stayed in groups of four, three weeks after weaning. One week later, the experimental male (BBS: $N = 24$, MvacATCC: $N = 21$) and female (BBS: $N = 24$, MvacATCC: $N = 18$) offspring were tested for general and social anxiety-related behaviors (SPAT; day 69 or 70) one day prior to the CAS procedure. The male and female offspring were randomly assigned to either the chronic stress group (males: chronic subordinate colony housing (CSC), BBS and MvacATCC: $N = 12$ per group; days 70-90, females: social instability paradigm (SIP), BBS: $N = 12$, MvacATCC: $N = 10$; days 71-120) or the no-stress control group (males: single-housed controls (SHCs), BBS: $N = 12$, MvacATCC: $N = 9$; days 70-90, females: group-housed controls (GHCs), BBS: $N = 12$, MvacATCC: $N = 8$; days 71-120). Notably, litter effects were minimized by ensuring that no more than $N = 3$ pups per sex and litter were

assigned to one experimental group (males: BBS-SHC: 9 litters; BBS-CSC: 6 litters; MvacATCC-SHC: 6 litters; MvacATCC-CSC: 5 litters; females: BBS-GHC: 10 litters; BBS-SIP: 6 litters; MvacATCC-GHC: 5 litters; MvacATCC-SIP: 5 litters). At the end of the respective CAS paradigm, all experimental mice were assessed for general anxiety-related behavior (OF/NO; day 89 or 119). On the day following the last behavioral assessment, all male and female offspring were euthanized and several physiological, bone-related and immunological parameters were determined. To check for alterations in the microbial α- and β-diversity, as well as for changes in the microbial community composition, fecal samples of MvacATCC- (N = 10), and BBS- (N = 10) dams at the time points of mating, birth and weaning, and respective CSC and SHC male offspring before and after chronic adult stress were collected (Schiele et al., 2026).

Table 9: Number of animals used in Experiment 1

Experiment 1	**BBS**		**MvacATCC**	
dams	10		10	
Experiment 1	**BBS**		**MvacATCC**	
male offspring	SHC N = 12	CSC N = 12	SHC N = 9	CSC N = 12
female offspring	GHC N = 12	SIP N = 12	GHC N = 8	SIP N = 10



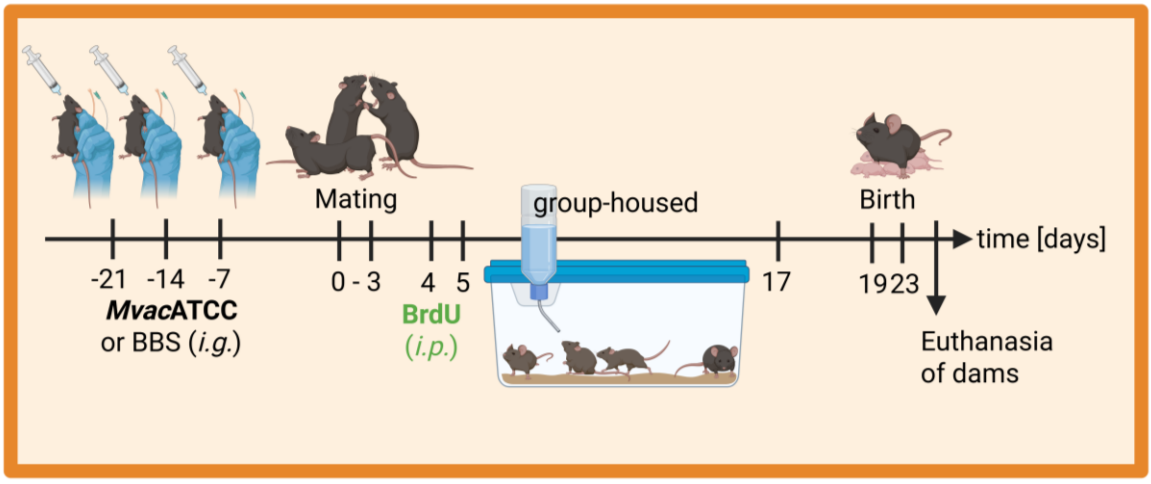
Timeline of Experiment 1 | Experimental Procedure. Adult, nulliparous female C57BL/6N mice received three weekly intragastric (*i.g.*) administrations of *Mycobacterium vaccae* ATCC 15483^{T} (*Mvac*ATCC) or its vehicle, borate-buffered saline (BBS), on days -21, -14, and -4 prior to mating with a conspecific breeding male conspecific (day 0-3). Following birth (between day 19 and 23) and weaning (between day 40 and 44; corresponding to postnatal day 21), adult male (blue box) and female (purple box) offspring were exposed to either chronic psychosocial stress (males: chronic subordinate colony housing, CSC; females: social instability paradigm, SIP) or respective control conditions (males: single-housed control, SHC; females: group-housed control, GHC). All experimental offspring were tested for social and general anxiety-related behaviors prior to (i.e., the social preference/avoidance test, SPAT; males: day 69, females: day 70) and after (i.e., open field/novel object test, OF/NO; males: day 89, females: day 119) chronic psychosocial stressor exposure. On the morning following the OF/NO test, male (day 90) and female (day 120) offspring were euthanized for the assessment of physiological, and immunological parameters.

Fecal samples were collected from dams at the start of mating (day 0), on the day of birth (between day 19 and 23), and at weaning (between day 40 and 44), as well as from adult male offspring before (day 69) and after (day 90) CSC exposure for fecal microbiome analysis. Adapted from Schiele et al. (2026), licensed under CC BY 4.0 and created in BioRender. Schiele, J. (2026) https://BioRender.com/h1ga64m.

Additionally, to investigate possible mechanisms underlying, *M. vaccae* ATCC 15483^{T} was repeatedly administered i.g. to a second group of nulliparous C57BL/6N female mice prior to mating, as in Experiment 1. In contrast, after the last day of mating, these females received a single intraperitoneal injection of bromodeoxyuridine (BrdU; 50 mg/kg in 0.9 % NaCl), as they served as controls to examine the effects of pregnancy stress on maternal hippocampal neurogenesis (this study is not part of the current thesis; **Timeline of Experiment 2**). These *M. vaccae* ATCC 15483^{T}- (N = 7) and BBS- (N = 5) treated dams, together with their respective offspring, were euthanized one to three days postpartum. Maternal mammary gland tissue was collected to determine whether the complement system, as part of the immune system, is crucial for the maternal-offspring transfer of potential *M. vaccae* ATCC 15483^{T} effects (Schiele et al., 2026).

Table 10: Number of animals used in Experiment 2

Experiment 2	BBS	MvacATCC
dams	N = 5	N = 7

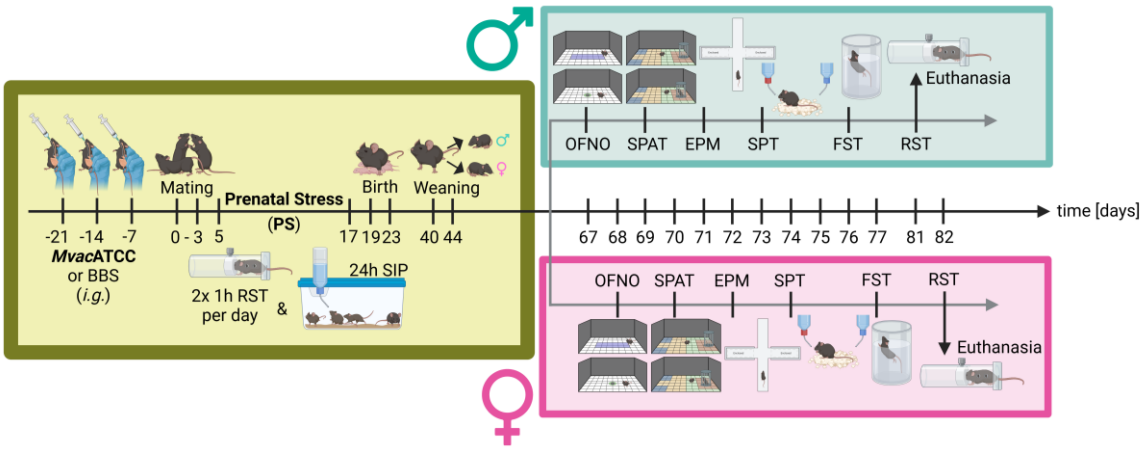


Timeline of Experiment 2 | Experimental Procedure. Adult, nulliparous female C57BL/6N mice received three weekly intragastric (*i.g.*) administrations of *Mycobacterium vaccae* ATCC 15483^{T} (*Mvac*ATCC) or its vehicle, borate-buffered saline (BBS), on days -21, -14, and -4 prior to mating with a conspecific breeding male (day 0-3). All pregnant females received a single intraperitoneal (*i.p.*) injection of bromodeoxyuridine (BrdU) on day 4 to investigate hippocampal neurogenesis during pregnancy (data not included in this thesis). During gestation, females were group-housed from day 5 to 17 and subsequently single-housed from day 17 onward. Following birth (between days 19 and 23), dams and their respective offspring were euthanized between lactation days 1 and 3. Mammary glands of dams were collected and snap-frozen to assess complement C3 expression. Adapted from Schiele et al. (2026), licensed under CC BY 4.0 and created in BioRender. Schiele, J. (2026) https://BioRender.com/v38buks.

To address **aim 2** (**Timeline of Experiment 3**), following *M. vaccae* ATCC 15483^{T} or BBS treatment and mating, as described in aim 1, pregnant females were subjected to PS, including restraint stress (twice per day for 1 h, at least 3 h apart) alternating with group-housing with three unfamiliar females for 24 h (SIP; days 4-16). Females in the noPS group were kept undisturbed in groups of four throughout the duration of the PS paradigm, serving as controls. After birth (day 19-23) and weaning (day 40-44), male (BBS-noPS, BBS-PS, MvacATCC-noPS and MvacATCC-PS: N = 12 per group) and female (BBS-noPS, BBS-PS, MvacATCC-noPS and MvacATCC-PS: N = 12 per group) offspring were tested every second day for general and social anxiety-related behaviors (OF/NO: day 67 or 68, SPAT: day 69 or 70, EPM: day 71 or 72), anhedonic-like behavior (SPT: day 73 or 74), and depressive-like behavior (FST: day 76 or 77). Changes in basal and acute stress-induced HPA axis (re)activity were tested on day 81 (males) and day 82 (females) by exposing all experimental mice individually to the RST (1 h). Subsequently, all PS- and noPS-exposed male and female offspring of dams treated with *M. vaccae* ATCC 15483^{T} and BBS were sacrificed as described above to assess physiological, immunological as well as bone-related parameters (Schiele et al., 2025).

Table 11: Number of animals used in Experiment 3

Experiment 3	**BBS**		**MvacATCC**	
dams	noPS N = 10	PS N = 9	noPS N = 10	PS N = 9
Experiment 3	**BBS**		**MvacATCC**	
male offspring	noPS N = 12	PS N = 12	noPS N = 12	PS N = 12
female offspring	noPS N = 12	PS N = 12	noPS N = 12	PS N = 12



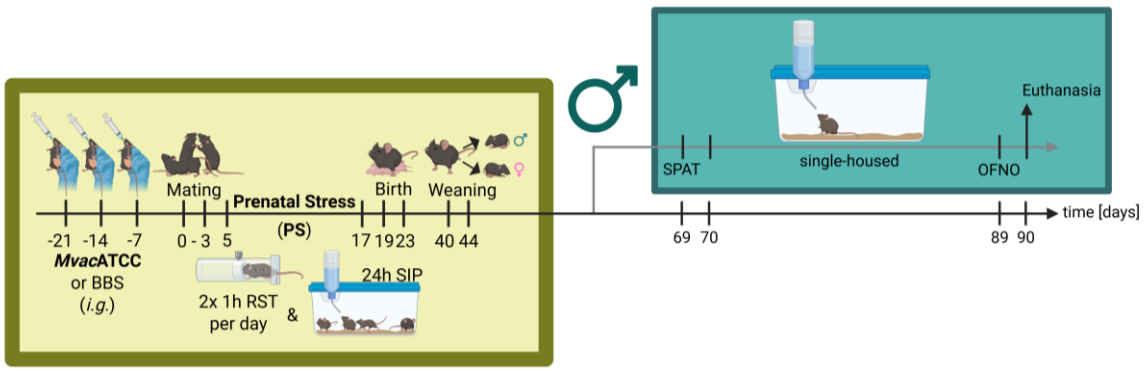
Timeline of Experiment 3 | Experimental Procedure. Adult, nulliparous female C57BL/6N mice received three weekly intragastric (*i.g.*) administrations of *Mycobacterium vaccae* ATCC 15483^{T} (*Mvac*ATCC) or its vehicle, borate-buffered saline (BBS), on days -21, -14, and -4 prior to mating with a conspecific breeding male (day 0-3). Unborn offspring of pregnant dams were exposed either to prenatal stress (PS, yellow box) between pregnancy days 5 and 17, consisting of alternating restraint stress (RST, 2x 1 h per day at least 3 h apart) and 24 h of social instability paradigm (SIP), or not (noPS, group-housed during the PS period).

Following day 17, pregnant females were single-housed to give birth between days 19 and 23. After weaning (days 40 – 44, corresponding to postnatal day 21), male (green box) and female (pink box) offspring were subjected to a battery of behavioral tests in alternating order: open field/novel object (OF/NO) test (males: day 67, females: day 68), social preference/avoidance test (SPAT; males: day 69, females: day 70), elevated plus maze (EPM; males: day 71, females: day 72), saccharin preference test (SPT; males: day 73, females: day 74), and forced swim test (FST; males: day 76, females: day 77). On days 81 and 82, male and female offspring, respectively, were exposed to an acute restraint stress test (RST) followed by immediate euthanasia. Physiological, bone-related, and immunological parameters were subsequently assessed in both sexes. Adapted from Schiele et al. (2025), licensed under CC BY 4.0 and created in BioRender. Schiele, J. (2026) https://BioRender.com/50tl6z6.

To determine whether PS exposure and intergenerational *M. vaccae* ATCC 15483^{T} administrations had long-lasting effects in the offspring, only single-housed male mice (Experiment 4; BBS-noPS and BBS-PS: $N = 12$ per group, MvacATCC-noPS: $N = 12$ and MvacATCC-PS: $N = 11$, **Timeline of Experiment 4**) were tested for general and social anxiety-related behaviors on day 69 (OF/NO) and day 89 (SPAT), respectively and were examined in terms of physiological, immunological, and bone-related changes on day 90 after euthanization (Schiele et al., 2025). The reason for examining only male offspring after day 90 was that significant changes in the main parameters of this study were observed only in males (day 81), but not females (day 82) (Schiele et al., 2025).

Table 12: Number of animals used in Experiment 4

Experiment 4	**BBS**		**MvacATCC**	
male offspring	noPS $N = 12$	PS $N = 12$	noPS $N = 12$	PS $N = 12$

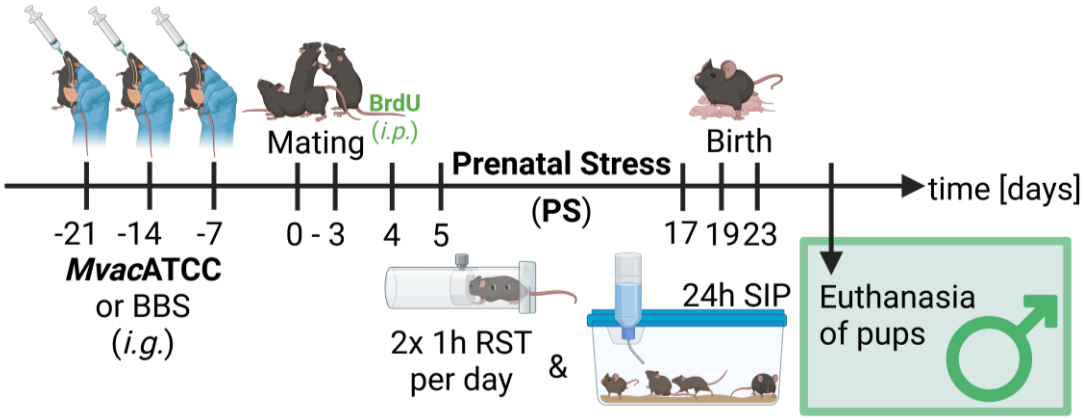


Timeline of Experiment 4 | Experimental Procedure. Adult, nulliparous female C57BL/6N mice received three weekly intragastric (*i.g.*) administrations of *Mycobacterium vaccae* ATCC 15483^{T} (*Mvac*ATCC) or its vehicle, borate-buffered saline (BBS), on days -21, -14, and -4 prior to mating with a conspecific breeding male (day 0-3). Unborn offspring of pregnant dams were exposed either to prenatal stress (PS, yellow box) between pregnancy days 5 and 17, consisting of alternating restraint stress (RST, 2 x 1 h per day at least 3 h apart) and 24 h of social instability paradigm (SIP), or not (noPS, group-housed during the PS period). Following pregnancy day 17, females were single-housed to give birth between days 19 and 23. After weaning (days 40 – 44, corresponding to postnatal day 21), male offspring (dark turquoise box) were single-housed from day 70 to day 90. Behavioral assessments were conducted before (social preference/avoidance test, SPAT; day 69) and afterwards (open field/novel object (OF/NO) test; day 89). All adult male mice were euthanized on day 90 for the assessment of physiological, bone-related, and immunological parameters. Adapted from (Schiele et al., 2025), licensed under CC BY 4.0 and created in BioRender. Schiele, J. (2026) https://BioRender.com/gvxuoih.

To investigate when immunological effects of prenatal stress first appear in male offspring, a third group of male pups was generated according to the procedure described in aim 1, Experiment 2 (**Timeline of Experiment 5**). Male offspring of dams treated with either BBS or *M. vaccae* ATCC 15483^{T} prior to mating were euthanized one to three days postpartum, and their whole spleens (i.e., $N = 2$ per litter) were collected to determine splenic IgG levels at the earliest possible time point (Experiment 5; BBS-noPS: $N = 10$, BBS-PS: $N = 14$, MvacATCC-noPS: $N = 15$, and MvacATCC-PS: $N = 13$).

Table 13: Number of animals used in Experiment 5

Experiment 5	**BBS**		**MvacATCC**	
male pups	noPS $N = 10$	PS $N = 14$	noPS $N = 15$	PS $N = 13$



Timeline of Experiment 5 | Experimental Procedure. Adult, nulliparous female C57BL/6N mice received three weekly intragastric (*i.g.*) administrations of *Mycobacterium vaccae* ATCC 15483^{T} (*Mvac*ATCC) or its vehicle, borate-buffered saline (BBS), on days -21, -14, and -4 prior to mating with a conspecific breeding male (day 0-3). All pregnant females received a single intraperitoneal (*i.p.*) injection of bromodeoxyuridine (BrdU) on day 4 to investigate hippocampal neurogenesis during pregnancy (data not included in this thesis). Unborn offspring of pregnant dams were exposed either to prenatal stress (PS) between pregnancy days 5 and 17, consisting of alternating restraint stress (RST, 2 x 1 h per day at least 3 h apart) and 24 h of social instability paradigm (SIP), or not (noPS, group-housed during the PS period). Subsequently, females were single-housed to give birth between days 19 and 23. Dams and their respective offspring were euthanized between lactation days 1 and 3. Offspring sex was determined by genotyping, and spleens of male pups were snap-frozen for the assessment of splenic immunological parameters. Adapted from (Schiele et al., 2025), licensed under CC BY 4.0 and created in BioRender. Schiele, J. (2026) https://BioRender.com/uj9a661.

3. Intragastric (i.g) administration of M. vaccae *ATCC15483^T or borate-buffered saline*

On days -21, -14, and -7, nulliparous female mice (Experiments 1-5) received three repeated administrations of heat-inactivated *M. vaccae* ATCC15483^T (100 µL; concentration: 1 mg/mL diluted in borate-buffered saline (BBS); final dose: 0.1 mg) or the corresponding volume of BBS prior to mating. All administrations were performed i.g. using a 20-gauge gavage needle (FST, Cat. No.: 18060-20). *M. vaccae* ATCC 15483^T was provided by the Institute of Medical Microbiology and Hygiene, Ulm University Medical Center, Germany and cultured at 37 °C and 5 % CO_2 until visible confluent growth was achieved. Subsequently, the RGMs were resuspended in phosphate-buffered saline and heat-inactivated for 30 min at 86 °C. Following centrifugation, the bacterial pellet was resuspended in its vehicle BBS and stored at -80 °C until heat inactivation was verified. Notably, the same dosage of the RGM has been employed in previous preclinical studies using i.g. (Langgartner et al., 2023a) or s.c. (Amoroso et al., 2020a; Reber et al., 2016b) administration of *M. vaccae* NCTC 11659 in male mice.

4. Mating, birth and weaning procedure

To generate experimental male and female offspring, two C57BL/6N female mice from either the *M. vaccae* ATCC 15483^T or the BBS group were group-housed with one breeder male mouse for a mating period of four days (days 0-3). At the end of mating day 4, females were returned to their original home cages and remained there until experimental day 17, when they were individually housed to provide a less stressful environment for the upcoming birth of the experimental offspring expected around pregnancy day 21 (days 19-23). If a litter was detected before 1400 h, that day was designated as postnatal day 0, if it was detected after 1400 h, the following day was designated as postnatal day 0. Litter size and total litter weight were assessed on postnatal day 1. Notably, two MvacATCC(-noPS)-dams lost their entire litter before weaning, therefore these animals were excluded from the maternal parameters “body weight per litter at weaning” and “number of male and female pups at weaning”.

5. Prenatal stress (PS) procedure

Following mating day 4, the chronic prenatal stress paradigm was applied for 13 consecutive days (days 4-16) using a PS model, as previously described (Hillerer et al., 2011) with minor modifications. During the light phase, pregnant females were alternately exposed to daily restraint stress (twice per day for 1 h, with at least 3 h between sessions) using restrainers (8.5 cm length and 3 cm diameter, equipped with ventilation holes) and to 24 h of social

instability, defined as group housing with four unfamiliar females in a standardized polycarbonate mouse cage (see housing conditions above). Both restraint stress and social instability are well-established potent stressors in female mice (Lu et al., 2021; Mazzari et al., 2023; Schmidt et al., 2010; Yohn et al., 2019). On days involving restraint stress, females were kept single-housed. From day 17 onward, pregnant mice were individually housed in preparation for delivery, as described above.

6. Observation of maternal behavior during first week after birth

During the first week after birth (postnatal days 1-6), excluding postnatal day 3 when the pup retrieval test was performed, maternal behavior was observed daily, as previously performed (Hillerer et al., 2011), with minor modifications. Between 0700 h and 1000 h, "on nest" behavior of the dam, defined as arched-back or blanket posture nursing, licking, grooming or carrying of pups, nest building, self-grooming and sleeping, was assessed in four observation intervals (each 5 sec long) within 10 min. "On nest" behavior was calculated as the percentage of observation intervals relative to the total number of observation intervals (72 intervals across 3 h), with the total set to 100%. Notably, two MvacATCC(-noPS)-treated dams lost their litters between birth and weaning and were therefore excluded from the analysis.

7. Pup retrieval test (PRT)

On postnatal day 3, maternal responsiveness was assessed in all dams using the PRT performed according to previously described procedures (Hillerer et al., 2012; Pardon et al., 2000). Briefly, litters were separated from their respective dams and placed in boxes on warm mats in a Scantainer for 1 h. The PRT was conducted in the home cage. However, a large portion of nesting material was removed to allow unobstructed visualization of the pups during recording. Following the 1 h separation period, a maximum of six pups were distributed in the four corners, along the walls, and in the center of the cage. The dam was then placed onto the remaining nest material. The procedure was video-recorded for 15 min to assess the latency to retrieve the first pup (sec) as well as the latency to place all pups back into the nest (sec). After completion of the PRT, the original nest material and the (remaining) pups of the litter were returned to the nest in the home cage. Notably, dams were excluded from the respective analyses due to non-performance in the PRT, failure to retrieve all pups to the nest or litter sizes smaller than $N = 6$ (pups: $N = 1$-3). Excluded animals are three BBS- and one MvacATCC-dam in Experiment 1, and one BBS-noPS, one BBS-PS and three MvacATCC-noPS dams in Experiment 3.

8. Open field/novel object (OF/NO) test

General anxiety-related behavior was assessed using the OF/NO test, as previously described (Amoroso et al., 2020a; Amoroso et al., 2020b; Foertsch et al., 2017b; Langgartner et al., 2023a; Langgartner et al., 2015). The testing arena (45 cm length x 27 cm width x 27 cm height; illumination: 350 lux) was subdivided into an inner zone (9 cm x 27 cm) and an outer zone. In the OF condition (5 min), the mouse was placed in the center of the arena and allowed to freely explore.

Subsequently, an unfamiliar round plastic object (diameter 3.5 cm, height 1.5 cm) was positioned in the center of the arena and the mouse was again allowed to explore the arena containing the novel object (5 min). Total distance moved during the OF exposure was analyzed as a measure of general locomotor activity. Anxiety-related behavior during the OF exposure was assessed by quantifying entries into the inner zone and the time spent in this zone. During the NO condition, entries into and the time in the direct contact zone surrounding the object (width: 1.625 cm) were recorded. Prior to each test, the arena was cleaned with water to remove odors and fecal residues from conspecifics. The software EthoVision XT (v11.5.1022; Noldus Information Technology, Wageningen, The Netherlands) was used to analyze all parameters listed above. Notably, in Experiment 1, one MvacATCC-GHC female jumped out of the arena during the recording of the NO condition and was therefore excluded from the analysis of NO parameters.

9. Social preference/avoidance test (SPAT)

The SPAT was conducted to assess general and social anxiety-related behavior, as previously described (Amoroso et al., 2020a; Amoroso et al., 2019; Langgartner et al., 2023a; Mazzari et al., 2023; Slattery et al., 2012). The same testing arena as used for the OF/NO test was employed. However, illumination was reduced to 20 lux. Following a 30 sec habituation period to the arena, an empty wire mesh cage (8.5 cm length x 7.5 cm width x 6.5 cm height) was introduced and presented to the experimental mouse for 150 sec (empty condition). This condition served to assess general anxiety-related behavior, as the empty cage represented a NO. Subsequently, the empty cage was replaced with an identical wire mesh cage containing an unfamiliar male or female C57BL/6N mouse, respectively. The experimental mouse was allowed to explore the arena containing the social stimulus (social condition) for an additional 150 sec. Time spent in the direct contact zone (width: 4.5 cm) surrounding the wire mesh cage was analyzed using the software EthoVision XT (v11.5.1022; Noldus Information Technology, Wageningen, The Netherlands).

Social preference was calculated as the time spent in the direct contact zone during the social condition divided by the total time spent in the direct contact zone across both the "empty" and "social" cage condition. As in the OF/NO test, the arena was cleaned prior to each SPAT session to remove odors and fecal residues from conspecifics.

10. Elevated plus maze (EPM) test

The EPM test was used as an additional behavioral test to assess general anxiety-related behavior. Testing was conducted as previously published (Amoroso et al., 2020a; Reber et al., 2007; Reber and Neumann, 2008). Testing of experimental mice was conducted between 0700 h and 1000 h. Each test session lasted 5 min and was performed in a designated behavioral box within the animal housing room. The plus maze consisted of two open arms (OA; 30 cm length, 6 cm width; illumination: 130 lux) and two closed arms (CA; 30 cm length, 6 cm width, 17 cm height; illumination: 30 lux), extending from a central square platform (6 cm x 6 cm) and elevated 130 cm above the floor. The edges of the open arms were 0.3 cm high to prevent mice from falling. Before the start of recording, each mouse was habituated for 30 sec in the same closed arm, with the entrance blocked to prevent further exploration. After each trial, the EPM was cleaned to remove odors and fecal residues from conspecifics. The parameters analyzed were the "number of entries into CA" and the "relative time spent in the OA", calculated as the percentage of time spent in the OA relative to the total time spent in all arms. EthoVision XT software was used for automated tracking and analysis.

11. Saccharin preference test (SPT)

The SPT is an established test to assess anhedonic-like behavior and was performed as previously described (Slattery et al., 2012). Prior to testing, all experimental mice were habituated to multiple drinking options by providing two drinking bottles filled with tap water for two days. During the 24 h SPT, male and female mice were single-housed and given free access to two bottles containing either a 0.05 % saccharin solution (Sigma-Aldrich, Chemie GmbH, Steinheim, Germany) or tap water. Bottle positions were exchanged according to a fixed schedule to prevent side bias. Before and after the SPT, the weight of both bottles was recorded and saccharin preference (%) was calculated using the following formula: (saccharin intake [g]/ total fluid intake [g]) x 100. Due to bottle leakage, one BBS-PS female and two MvacATCC-noPS females were excluded from the analysis.

12. Forced swim test (FST)

The FST is an established test to assess depression-like behavior and was performed as previously described (Bai et al., 2001; Langgartner et al., 2023b; Slattery et al., 2012). Testing was conducted between 0700 h and 1000 h under an illumination of 200 lx. Each mouse was individually placed into a transparent plastic cylinder (40 cm height, 12 cm diameter) filled with water (13 cm depth; 23 ± 2 °C) for a total duration of 6 min. EthoVision XT software was used to record and analyze the time spent mobile at a velocity > 1.7 cm/ sec during the last 4 min of the test. After each testing session, the cylinder was refilled with fresh water. To assess depression-like behavior, time spent immobile was calculated as 240 sec minus time spent mobile [sec].

13. Restraint stress test (RST)

The acute RST was performed to assess alterations in the acute stress response following prenatal stress exposure and was conducted as previously published (Chen and Herbert, 1995; Heinrichs and Koob, 2006). Experimental male and female mice were individually restrained for 1 h in a cylindrical plastic tube (8.5 cm length, 3 cm diameter) with ventilation holes along the walls. Restraint was performed between 0700 h and 1000 h prior to euthanasia (Experiment 3).

14. Chronic subordinate colony housing (CSC) paradigm

The CSC paradigm was performed as previously published (Langgartner et al., 2023a). Adult male mice were weighted and, based on bodyweight, subjected to either the stress (CSC) or the no-stress single-housed control (SHC) group. Chronic psychosocial stress was induced by co-housing four C57BL/6N male mice with a dominant, aggressive CD-1 male mouse (resident of the experimental cage) for 21 consecutive days. To prevent habituation and to maintain a high level of psychosocial stress, the aggressive CD-1 male was exchanged on days 8 and 15. Because this stress model is accompanied by physical confrontations between the dominant resident and experimental mice, body weight and bite wounds were assessed twice per week. Prior to the start of the CSC paradigm, potential aggressor mice were tested for aggressiveness to exclude individuals that severely attacked or bit their conspecifics. During the first hour after experimental colony formation on days 1, 8, and 15, mice were monitored to confirm subordinate behavior and establishment of social hierarchy. As group-housing itself has been shown to be stressful and to induce alterations comparable to chronic stress effects, single housing in male mice represents an appropriate control condition in chronic psychosocial stress experiments (Kappel et al., 2017; Singewald et al., 2009).

Accordingly, SHCs remained undisturbed in their home cages for 21 days, except for routine weight monitoring and bedding changes.

15. Social instability paradigm (SIP)

The SIP is an animal model used to induce chronic psychosocial stress in adult C57BL/6N female mice (Schmidt et al., 2010; Sterlemann et al., 2008; Yohn et al., 2019) and was performed as previously described (Mazzari et al., 2023). Female mice were exposed to group-housing with up to three unfamiliar females for seven consecutive weeks. Cage mate composition was changed every third day. To minimize repeated encounters with the same cage mates, cage composition was randomized using the online version of GraphPad "Assign subjects to group" (GraphPad Software, San Diego, CA, USA). Body weight and bite wounds were assessed every third day during cage composition changes. In females, group-housing of three to four animals per cage was used as the respective control condition, with group-housed control (GHC) mice remaining undisturbed with their cage mates, except for routine body weight monitoring and bedding changes.

16. Blood collection at RST and after in vivo experiment

During the first 3 min of the RST, representing baseline CORT levels, tail blood was collected from conscious male and female mice (Experiment 3) into ethylenediaminetetraacetic acid (EDTA)-coated tubes (Sarstaedt, Nuembrecht, Germany). At the end of the respective experiments (Experiments 1 and 3), trunk blood was collected into EDTA-coated tubes (Sarstaedt, Nuembrecht, Germany), following brief CO_2 inhalation and decapitation, as previously described (Amoroso et al., 2019; Amoroso et al., 2020b). For further analysis, samples were centrifuged at 4 °C for 10 min at 5000 g and plasma from each mouse was stored at -20 °C.

17. Collection of organs and weight determination

After euthanasia, stress-sensitive organs, such as adrenal glands, thymus, and spleen, as well as long bones, including femur and tibia, were removed from each mouse and carefully pruned of fat or muscle tissue, respectively. The weights of the adrenal glands, thymus, and spleen were determined. During organ preparation in Experiment 3, the adrenal glands of one BBS-noPS male, one BBS-PS male, and one MvacATCC-noPS female were cut. Therefore, these mice were excluded from statistical analysis of the respective parameter. Spleens from male and female mice in Experiments 1 and 4 were stored in ice-cold Hanks' balanced salt solution (HBSS; Sigma-Aldrich), whereas spleens from male mice in Experiments 4, as well as both spleens and tails from male pups in Experiment 5, were frozen at -80 °C.

Bones of the right hind limb from experimental mice in Experiments 1, 3 and 4 were dissected, cleaned and stored in 4 % formalin at 4 °C.

18. Bite wound severity assessment

In male mice exposed to CSC (Experiment 1), the severity of bite wounds was assessed using an established bite score as previously described (Foertsch et al., 2017a; Kempter et al., 2021). The skin, with fur attached, was removed in such a way that it remained connected to the tail root and hind limbs. Subsequently, standardized pictures of the skin (dermal layer) and the corresponding body (subdermal layer) were taken from all CSC mice using identical positioning and camera settings.

Each image was digitally edited by overlaying a standardized grid consisting of 20 squares (4 x 5 squares; each square includes 180 x 240 pixels; overall 720 x 1200 pixels), covering the skin and an identical grid covering the body. Bite wound severity was then assessed according to the established scoring system, including the evaluation of size and intensity of bite injuries in both dermal (skin) and subdermal (body) layers:

Dermal scoring:

- Area affected: 0 = no bites, 1 = single bites, 2 = several bites, 3 = spacious bites, 4 = bites in the entire square (maximum subscore: 80).
- Intensity of bite wounds: 1 = mild, 2 = moderate, 3 = severe, 4 = necrotic (maximum subscore: 80).
- Degree of purulence: 0 = no purulence, 1 = mild purulence, 2 = severe purulence (maximum subscore: 40).

Subdermal scoring:

- Area affected: 0 = no bites, 1 = < 1/3 of the area, 2 = 1/3-2/3 of the area, 3 = > 2/3 of the area (maximum subscore: 60).
- Intensity of bite wounds: 1 = mild, 2 = severe (maximum subscore: 40).

The total bite score ranges from 0 to 300 and represents the sum of dermal and subdermal subscores. Bite scoring was performed by an investigator blinded to the experimental groups.

19. Sex determination via polymerase chain reaction (PCR) in pups of BrdU-injected dams

To determine the sex of pups from Experiment 5, tail clippings were used as a source material for a simplex polymerase chain reaction (PCR) assay (KAPA2G Fast Hot Start Genotyping Mix, Thermo Fisher Scientific) as previously described (Clapcote and Roder, 2005; Yotova et al., 2024).

Genomic DNA was amplified from tail tissue samples using the following primers; forward: 5'-CTGAAGCTTTTGGCTTTGAG-3', reverse: 5'-CCACTGCCAAATTCTTTGG-3'. The primer sequences did not match the X-chromosome-specific gene *Jarid 1c* (Ensembl Transcript ID: ENSMUST00000082177), as well as the Y-chromosome-specific gene *Jarid 1d* (Ensembl Transcript ID: ENSMUST00000055032). A single band at 331 base pairs (bp) indicated female sex, whereas two bands at 302 bp and 331 bp indicated male sex.

20. Femoral and tibia length measurement

The length of the right tibia in male and female mice from Experiment 1 and the length of the right femur in male and female mice from Experiments 3 and adult male mice from Experiment 4 were measured using a digital precision caliper, as described previously (Gebauer et al., 2025). One male per group in Experiment 4 was excluded from femoral length measurements due to technical issues.

21. Femoral µCT analysis

µCT analysis was performed on right femurs from male and female mice in Experiment 3 and from adult males in Experiment 4 (Foertsch et al., 2017b; Gebauer et al., 2025). Scans were conducted using a Skyscan 1172 scanning system (Skyscan, Kontich, Belgium) with the following settings: 50 kV, 200 mA, and a voxel size of 8µm. Data were analyzed using Skyscan software packages NRecon, Data Viewer CTAn, and CTVox in accordance with the ASBMR guidelines (Bouxsein et al., 2010). Tissue mineral density (TMD) was determined by including two hydroxyapatite (HA) calibration phantoms with densities of 250 and 750 mg/cm^3 in each µCT scan. Trabecular and cortical bone parameters were assessed within predefined volumes of interest (VOI) as previously described (Foertsch et al., 2017b). The trabecular VOI in the metaphyseal region was defined starting 200 µm proximal to the growth plate and extended over a length of 280 µm. The cortical VOI spanned from 80 µm proximal to 80 µm distal relative to the center of the diaphysis. Thresholds of 642 mg HA/cm^3 for cortical bone and 394 mg HA/cm^3 for trabecular bone were applied to distinguish mineralized from non-mineralized tissue. Due to technical issues, the following males from Experiment 4 were excluded from µCT analysis: two BBS-noPS, one BBS-PS, two MvacATCC-noPS, and one MvacATCC-PS for trabecular (Tb) parameters (bone volume to tissue volume ratio (BV/TV), tissue mineral density (TbTMD), thickness (TbTh), number (TbN), and separation (TbSp)), and one BBS-noPS, three MvacATCC-noPS, and one MvacATCC-PS for cortical (c) parameters (cTMD and cTh).

22. Plasma CORT enzyme-linked immunoprecipitation assay (ELISA)

Plasma CORT levels were determined using a commercially available ELISA kit (IBL International GmbH, Hamburg, Germany), performed according to the manufacturer's instructions and as previously described (Langgartner et al., 2018a; Reber et al., 2011). Briefly, samples in EDTA-coated tubes were centrifuged (5000 g, 10 min, 4 °C), and the supernatant was transferred into new tubes and stored at -20 °C until further processing. Before starting the plasma CORT ELISA, all reagents were brought to room temperature and wells were washed using the prepared wash buffer. Meanwhile, samples, standards (in duplicates), and controls (on every plate the same control was used for later adjustment) were prepared and plated. Plasma samples collected at the basal time point were diluted 1:10 (lowest standard: 1.732 ng/ mL), whereas plasma samples obtained after the 60 min of acute restraint stress were diluted 1:30 (lowest standard: 1.732 ng/ mL). Subsequently, 200 µL of enzyme conjugate was added to all wells, the plate was covered with aluminum foil, and incubated for 1 h at room temperature. After discarding the solutions and performing four washing steps using the prepared wash solution, 100 µL substrate solution was added to all wells, followed by incubation for 15 min at room temperature. The enzymatic reaction was terminated by adding 50 µL of stop solution. The optical density (OD) was measured at 450 nm using the Fluostar Optima plate reader (BMG Labtech GmbH, Offenburg, Germany), and the OPTIMA software was used to generate a standard curve and the corresponding raw values. Notably, three BBS-noPS males, one MvacATCC-noPS male, one MvacATCC-noPS female, and one MvacATCC-PS female were excluded due to insufficient volume of plasma for ELISA analysis.

23. Assessment of chemotactic activity of neutrophil/ PMN-MDSCs-enriched WBCs

The chemotactic assay of neutrophil/ PMN-MDSC-enriched WBC was assessed as described previously (Kempter et al., 2023) with modifications. Briefly, trunk blood was collected in EDTA-coated tubes (Sarstaedt, Nuembrecht, Germany) and centrifuged (400 g, 5 min at room temperature) to remove plasma. The remaining EDTA blood was gently resuspended in saline (NaCl 0.9 %) and pooled per experimental group before being layered onto Ficoll (GE Healthcare, Chicago, USA) in 15 mL Falcon® conical tubes (Corning Life Sciences, Kaiserslautern, Germany). Samples were centrifuged at 400 g for 30 min at room temperature using the lowest level of acceleration setting without deceleration. Subsequently, the supernatant containing plasma, monocytes and Ficoll layer was removed. The pellet was resuspended in saline and mixed with dextran (Sigma-Aldrich, St. Louis, MO, USA) and 5 % NaCl.

Dextran sedimentation was performed for 45 min, after which the supernatant was transferred to a new 15 mL Falcon® tube and centrifuged (400 g, 5 min, at room temperature). The supernatant was discarded, and erythrocytes were lysed using Ampuwa H_2O (Fresenius, Bad Homburg, Germany). Lysis was terminated by adding 2.7 % NaCl, followed by a final centrifugation step (400 g, 5 min, at room temperature). The supernatant was discarded, and remaining cells were resuspended in phosphate-buffered saline (PBS; Gibco, Carlsbad, CA, USA) supplemented with 0.1 % bovine serum albumin (BSA). Cells were counted using a ViCell CR cell counter (Beckmann Coulter, Krefeld, Germany) and adjusted to a concentration of 8 x 10^4 cells/ 44 µL. The adjusted cells were then labeled with 2',7'-bis-(2-carboxyethyl)-5-(6)-carboxyfluorescein (BCECF) for 30 min at 37 °C in an incubator, followed by centrifugation and resuspension in HBSS (Gibco, Carlsbad, CA, USA) supplemented with 0.1 % BSA. Chemotactic activity was assessed using a 96-well chemotaxis mini-chamber (Neuroprobe, Cabin John, MD, USA). A total of 33µL of N-formylmethionine-leucyl-phenylalanine (fMLP; 10^{-2} M stock solution diluted to a 10^{-6} M working solution) was loaded into wells of the lower chamber as chemoattractant. The lower and upper chamber were separated by a polycarbonate membrane with pore size of 3 µm (Neuroprobe, Cabin John, MD, USA). Subsequently, 44 µL of cell suspension were added to wells of the upper chamber in six technical replicates per experimental group, followed by incubation of the assembled chamber for 30 min at 37°C. After removal of the membrane, chemotactic activity was quantified by measuring the fluorescence of migrated BCECF-labelled cells on the membrane using a Fluostar Optima plate reader (BMG Labtech GmbH, Offenburg, Germany) and OPTIMA software. As samples from adult males (Experiments 1 and 4) were analyzed in two experimental rounds, values obtained in the second round were adjusted to those of round 1 using a correction factor calculated from the mean of the six technical replicates of the MvacATCC-noPS group measured in both rounds.

24. Analysis of plasma LBP concentrations

Plasma LBP concentrations in experimental male and female mice from Experiment 3 were determined using a mouse LBP ELISA kit (HycultBiotech, Uden, Netherlands). The assay was performed according to the manufacturer's instructions. Briefly, all reagents were brought to room temperature (20 – 25 °C) prior to use. The prepared plate was washed four times with wash buffer using an automated plate washer before 100 µL of samples, controls, and standards (in duplicates) were added to the appropriate wells. The plate was covered and gently tapped to remove air bubbles, followed by 1 h of incubation at room temperature.

After incubation, the washing step was repeated, and 100 µL of diluted tracer solution was added to each well. The plate was covered and incubated for 1 h. at room temperature). Another washing step followed. Subsequently, 100 µL of diluted streptavidin-peroxidase was added to each well, and the plate was covered and incubated for an additional 1 h at room temperature. After washing, 100 µL of TMB substrate was added to each well. The plate was protected from direct sunlight by covering it with aluminum foil. Following an incubation time of 30 min, the reaction was terminated by adding 100 µL of stop solution to the wells. LBP concentrations were quantified by measuring absorbance at 450 nm using a microplate reader (Fluostar Optima, BMG Labtech GmbH, Offenburg, Germany).

25. Ex vivo isolation of splenocytes

Ex vivo isolation of splenocytes was performed as previously described (Foertsch et al., 2017a; Foertsch et al., 2020; Kempter et al., 2023; Langgartner et al., 2023a). Whole spleens were mechanically homogenized by pressing them through a 70 µm cell strainer (Corning Life Sciences, USA) using the plunger of a syringe into 50 mL Falcon® tubes (Corning Life Sciences, USA). Following centrifugation (512 g, 12 min, at 4 °C), erythrocytes were lysed using a lysis buffer containing 155mM NH_4Cl, 10 mM $KHCO_3$, 10 mM EDTA. Lysis was terminated after 2 min by adding HBSS supplemented with 0.1 % heat-inactivated fetal calf serum (FCS). The cell suspension was subsequently filtered through a 70 µm nylon cell strainer, which was rinsed with additional buffer. Cell numbers were determined using a ViCell CR cell counter (Beckmann Coulter, Krefeld, Germany), and splenocytes were adjusted to a concentration of 5 x 10^6 cells/ mL in Roswell Park Memorial Institute (RPMI) medium supplemented with 10 % FCS, 50 U/ mL penicillin and 50 µg/ mL streptomycin (RPMI supplemented). Freshly isolated cells were either used immediately for the splenic GC sensitivity assay, pelleted and snap-frozen for storage at -80 °C for later protein isolation and western blot analysis, or resuspended in freezing medium consisting of FCS and dimethyl sulfoxide (DMSO; C_2H_6OS Carl Roth GmbH + Co. KG, Karlsruhe, Germany; diluted 1:10) and stored at -80 °C for later flow cytometry analysis.

26. Functional ex vivo GC sensitivity assay of isolated splenocytes

Following *ex vivo* splenocyte isolation, spleen cells were stimulated with LPS and CORT to assess functional *in vitro* GC sensitivity. The assay was performed as described previously (Foertsch et al., 2017a; Foertsch et al., 2020; Kempter et al., 2023; Langgartner et al., 2023a). Briefly, 2.5 x 10^5 isolated spleen cells per well were either stimulated with LPS (Escherichia coli O111:B4; final concentration 1.0 µg/ mL, Sigma-Aldrich, Deisenhofen, Germany) or

left untreated to determine background activity (basal condition). GC sensitivity was assessed by treating both basal and LPS-stimulated splenocytes with different concentrations of CORT diluted in 95 % ethanol, resulting in final well concentrations of 0 µM, 0.1 µM and 5 µM. Cells were cultured in flat-bottom 96-well plates at a final volume of 100 µL per well and incubated for 24 h at 37 °C in an incubator with 5 % CO_2. After overnight incubation, cell viability was assessed using a commercially available colorimetric assay (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA). Absorbance (optical density; OD) was measured at 450 nm using an ELISA plate reader (Fluostar Optima, BMG Labtech GmbH, Offenburg, Germany). Measured values were corrected using blank controls, and delta cell viability was calculated by subtracting the OD of basal splenocytes from the corresponding LPS-stimulated well. The delta OD obtained under the CORT 0 µM condition was set to 100 %. Based on these calculations, high GC sensitivity was defined as significantly reduced cell viability in the presence of CORT, whereas low GC sensitivity was defined as high delta cell viability despite CORT treatment. GC resistance was defined as significant increase in delta cell viability in presence of CORT independent of the experimental factor (stress or treatment). Due to technical issues, one female from the BBS-GHC group and one female from the MvacATCC-SIP group in Experiment 1, as well as one BBS-noPS male in Experiment 4 were excluded from statistical analysis (delta cell viability, CORT 0 µM set to 100 %).

27. Protein extraction of splenocytes

Protein extraction from pelleted and snap frozen splenocytes obtained from male mice in Experiment 4 was performed as previously described (Magdeburg et al., 2012; Wu et al., 2010). Splenocytes were resuspended in an appropriate volume of cell extraction buffer (CEB, Invitrogen, Waltham, MA, USA) at a ratio of 1 mL per 10^8 cells, supplemented with complete protease inhibitor (Roche Diagnostics, Penzberg, Germany). Samples were subsequently incubated on ice and vortexed intermittently every 10 min for a total of 30 min. Thereafter, samples were centrifuged at 247 g for 10 min at 4 °C, and the protein-containing supernatant collected and stored at -80 °C until further analysis.

28. Protein extraction of total spleen

Spleens obtained from male and female mice in Experiment 3 and from male pups in Experiment 5 were snap-frozen and protein isolation of splenic tissue was performed as described previously (Lu et al., 2010).

Spleens of Experiment 3 animals were bisected and weighed prior to extraction. Proteins were extracted using the Tissue Extraction Reagent I (Invitrogen, Carlsbad, CA, USA), which was supplemented beforehand with Complete MINI Protease Inhibitor (Roche Diagnostics, Penzberg, Germany). Subsequently, extraction buffer was added in a ratio of 10 mL per 1 g splenic tissue. Samples were mechanically homogenized, followed by centrifugation (10000 g, 5 min). The protein-containing supernatant was collected and stored at -80 °C until further analysis.

29. Assessment of splenic TLR2 and TLR4 expression levels using western blot

Following protein extraction, splenic TLR2 and TLR4 expression levels in experimental male and female mice from Experiment 1 were assessed using western blot analysis as previously described with minor modifications (Langgartner et al., 2023a). The Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Scientific, Rockford, USA) was used to determine total protein concentrations. Equal amounts of protein lysates (15 µg per sample) were loaded onto 4-20 % Mini-PROTEAN TGX Stain-free Gel (Bio-Rad Laboratories, Munich, Germany). The detection of whole protein was performed using a Molecular Imager® ChemiDoc™ MP system (Bio-Rad Laboratories, Munich, Germany), followed by transfer of proteins onto nitrocellulose blotting membranes (GE Healthcare Life Sciences, Germany). Membranes were blocked with 5 % BSA in Tris-buffered saline containing Tween (TBS-T) and subsequently incubated overnight at 4°C on a shaker with a primary anti-TLR4 antibody diluted 1:1000 (Santa Cruz Biotechnology, Dallas, TX, USA). After washing, membranes were incubated with an HRP-conjugated goat anti-mouse secondary antibody diluted 1:10000 (Jackson Immuno Research, Cambridgeshire, UK) for 1 h at room temperature. Signal detection was performed using Clarity™ Western ECL substrate (Bio-Rad, Laboratories, Munich, Germany), and antibody binding was visualized using the Molecular Imager® ChemiDoc™ MP system (Bio-Rad Laboratories, Munich, Germany). Semiquantitive densitometric analysis was conducted using the Image Lab™ Software (Bio-Rad Laboratories, Munich, Germany). Membranes were then stripped using re-Blot Plus Antibody Stripping Solution (Millipore GmbH, Schwalbach, Germany), re-blocked with 5 % milk powder in TBS-T, and incubated overnight at 4 °C with a primary antibody for goat anti-TLR2 diluted 1:200 (R&D Systems, Minneapolis, MN, USA). Subsequently, membranes were incubated with HRP-conjugated chicken anti-goat secondary antibody diluted 1:1000 (R&D Systems, Minneapolis, MN, USA) for 1 h at room temperature. Signal detection and densitometric analysis were performed as described above.

For each mouse, TLR4 (~100 kDa) and TLR2 (~96 kDa) protein expression levels were normalized to whole protein. Due to technical issues, two female mice from the MvacATCC-GHC group were excluded from both TLR2 and TLR4 protein expression analyses.

30. Assessment of splenic IgG expression levels using western blot

Following protein extraction from either whole spleens (male and female mice from Experiment 3 and male pups from Experiment 5) or isolated splenocytes (male mice Experiment 4), splenic IgG expression levels were assessed by western blot analysis performed as previously described (Foertsch et al., 2017a; Langgartner et al., 2023a; Langgartner et al., 2017), with minor modifications. BCA assay was used to determine protein concentrations in respective samples. Samples were diluted to obtain a protein amount of 20 µg per sample and subsequently loaded onto 4-20 % Mini-PROTEAN TGX Stain-Free gels (Bio-Rad Laboratories, Munich, Germany). Protein separation was performed at 60 V for 30 min, followed by 120 V for 1 h and 15 min. The detection of the whole protein amount was conducted using a Molecular Imager® ChemiDoc™ MP system (Bio-Rad Laboratories, Munich, Germany). Proteins were then transferred onto nitrocellulose blotting membranes (GE Healthcare Life Sciences, Germany) using a semi-dry blotting system for 50 min at 15 V in transfer buffer. Membranes were blocked for 1 h at room temperature in TBS-T containing 4 % BSA (fraction V, Serva, Heidelberg, Germany), while shaking to avoid nonspecific antibody binding. Subsequently, membranes were incubated for 1 h at room temperature on a shaker with an HRP-linked anti-mouse IgG antibody (Cell Signaling Technology, Danvers, USA). Afterwards, membranes were washed three times for 10 min in TBS-T, followed by a final washing step for 5 min in TBS. Signal detection was performed using Clarity™ Western ECL substrate (Bio-Rad Laboratories, Munich, Germany), and antibody binding was visualized using the Molecular Imager® ChemiDoc™ MP system. Semiquantitative densitometric analyses were conducted using Image Lab™ Software (Bio-Rad Laboratories, Munich, Germany). For each mouse, IgG protein expression was normalized to the amount of whole protein, and gel-to-gel variation was controlled by normalization to a reference control sample included on every gel.

31. Preparation of cell-protected frozen splenocytes for flow cytometry

Splenocytes were first completely thawed and resuspended in RPMI-supplemented medium. After centrifugation and a subsequent washing step with RPMI-supplemented, cells were counted using a ViCell CR cell counter (Beckman Coulter, Krefeld, Germany).

Each sample was then adjusted to a final concentration of 5 x 10^6 cells per mL using the appropriate volume of RPMI containing 10 % FCS and penicillin/streptomycin.

32. Flow cytometry analysis of splenic myeloid subpopulations

The percentages of $CD11b^+Ly6C^+Ly6C^-$ monocytes/MO-MDSCs, $CD11b^+Ly6C^-Ly6C^+$ neutrophils and $CD11b^+Ly6C^+Ly6C^+$ PMN-MDSCs in isolated splenocytes were analyzed as previously described (Langgartner et al., 2023a). Fluorescently labelled antibodies against CD11b (APC-eFluor™ 780), Ly6C (APC), and Ly6G (V450) (all from BD Bioscience, Franklin Lakes, NJ, USA) were used to stain a total of 1 x 10^6 splenocytes at 4 °C for 30 min. Subsequently, cells were washed using flow-associated cell sorting (FACS) buffer (PBS containing 10 % FCS and 0.1 % NaN_3), followed by fixation using the FoxP3 Transcription Factor Staining Buffer Set (eBioscience, Carlsbad, CA, USA). After two additional washing steps with FACS buffer, cells were resuspended in FACS buffer and analyzed with a LSR II flow cytometer (BD Bioscience, Heidelberg, Germany). Data analysis was performed using FlowJo™ software (Version 10; Tree Star, Ashland, OR, USA).

33. Flow cytometry analysis of splenic Tregs

The percentages of $CD4^+CD25^+FoxP3^+$ Tregs in isolated splenocytes were analyzed as previously described (Langgartner et al., 2023a). Fluorescently labelled antibodies against CD4 (clone RM4-5, APC-eFluor™ 780), CD25 (clone PC61.5, PE) (both from Thermo Fisher Scientific, Waltham, MA, USA) were used to stain a total of 1 x 10^6 splenocytes at 4 °C for 30 min. Subsequently, cells were washed using flow-associated cell sorting (FACS) buffer (PBS containing 10 % FCS and 0.1 % NaN_3), followed by fixation using the FoxP3 Transcription Factor Staining Buffer Set (eBioscience, Carlsbad, CA, USA). Following, splenocytes were stained at 4 °C for 30 min with an antibody for the intracellular marker FoxP3 (clone FJK-16s; Thermo Fisher Scientific, Waltham, MA, USA). After one additional washing step with FACS buffer, cells were transferred into FACS buffer and analyzed with a LSR II flow cytometer (BD Bioscience, Heidelberg, Germany). Data analysis was performed using FlowJo™ software (Version 10; Tree Star, Ashland, OR, USA).

34. $CD4^+CD25^+$ Treg isolation from splenocytes and Treg suppression assay

To assess the suppressive capacity of $CD4^+CD25^+$ activated Tregs, these cells were first isolated from pooled single-cell suspensions of splenocytes per experimental group using the $CD4^+CD25^+$ Regulatory T Cell Isolation Kit (Miltenyi Biotec Inc., San Jose, CA, USA), according to the manufacturer's instructions for magnetic-activated cell sorting (MACS) separation under sterile conditions.

Briefly, the single-cell suspension of splenocytes was strained through a 30 µm pre-separation filters (Miltenyi Biotec Inc., CA, USA) into a 15 mL Falcon® conical tube (Corning life, USA). The cells were prepared for a cell count by means of the automatic ViCell CR cell counter (Beckman Coulter, CA, USA) and adjusted to 20 x 10^6 cells per mL. Subsequently, cells were centrifuged at 300 g for 10 min at 4°C. The supernatant was discarded and the cell pellet was resuspended in MACS buffer (phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA) and 2mM EDTA), prepared by diluting MACS BSA Stock Solution (Miltenyi Biotec Inc., CA, USA) 1:20 with autoMACS Rinsing Solution (Miltenyi Biotec Inc., CA, USA).

$CD4^+CD25^+$ Tregs were isolated in a two-step procedure.

First, non-$CD4^+$ T cells were indirectly magnetically labeled using $CD4^+CD25^+$ Regulatory T Cell Biotin-Antibody Cocktail and incubated for 15 min at 2-8°C. Subsequently, MACS buffer, Anti-Biotin MicroBeads (conjugated to a monoclonal anti-biotin antibody; isotype: mouse IgG1), and CD25-PE antibody (monoclonal anti-mouse CD25 antibody conjugated to R-Phycoerythrin (PE); isotype: rat IgM) were added. After thorough mixing, the suspension was incubated for 15 min at 2-8°C. Magnetically labeled cells were then depleted by the magnetic separation over LD Columns (Miltenyi Biotec Inc., CA, USA; maximum 5 x 10^8 total cells) placed in the magnetic field of a QuadroMACS™ Separator (Miltenyi Biotec Inc., CA, USA). LD Columns were prepared by rinsing with MACS buffer before applying cell suspension. Non-$CD4^+$ T cells were retained in the column, whereas unlabeled $CD4^+$ T cells were collected in the flow-through. The retained non-$CD4^+$ T cells were subsequently flushed out of the column into a separate 15 mL Falcon® tube by rinsing with MACS buffer.

Next, magnetic labeling of the $CD25^+$ T cells was performed. The $CD4^+$ T cell suspension was centrifuged at 300 g for 5 min at 4°C, the supernatant was discarded, and the cell pellet was resuspended in MACS buffer. Anti-PE MicroBeads (conjugated to monoclonal anti-PE antibodies; isotype: mouse IgG1) were added and incubated for 15 min at 2-8°C.

For the positive selection of $CD4^+CD25^+$ activated T cells, MS Columns (Miltenyi Biotec Inc., CA, USA; maximum 2 x 10^9 total cells) placed in the magnetic field of an OctoMACS™ Separator (Miltenyi Biotec Inc., CA, USA) were used. After preparation of the columns as described above, the CD4+ cell fraction was applied to the column. Magnetically labeled $CD4^+CD25^+$ cells were retained in the column, while $CD4^+CD25^-$ cells passed through. At this stage, the collected $CD4^+CD25^-$ cells were prepared for storage at -80 °C. To increase purity, a second column run was performed. After removal from the magnetic separator, the MS Column was placed onto a new MS Column in the magnetic field of the OctoMACS™

Separator and washed with MACS buffer. The positively selected $CD4^+CD25^+$ cells passed through the second column. Finally, the remaining cell fraction of interest was eluted by flushing the column with MACS buffer using the supplied plunger. After completion of the $CD4^+CD25^+$ T cell isolation, the cells were again counted using a ViCell CR cell counter (Beckman Coulter, Krefeld, Germany) and adjusted to a concentration of 1.2 x 10^6 cells per mL. Furthermore, responder cells were isolated from single-cell suspension, counted, and adjusted to 5 x 10^6 cells per mL as described above in the section "*Ex vivo isolation of splenocytes*". Experiment 1 was performed in two rounds. In round 1, pooled spleen cells from experimental male (BBS-SHC) or female (BBS-GHC) mice were used, whereas in round 2, spleen cells from a non-experimental breeding male or female were used, respectively. Responder cells were subsequently labeled with carboxyfluorescein succinimidyl ester (CFSE) using the CellTrace™ CFSE Cell Proliferation Kit for flow cytometry (Invitrogen, USA) according to the manufacturers protocol. For this purpose, responder cells were transferred into 50 mL Falcon® tubes and centrifuged at 572 g for 10 min at 4°C. A CFSE stock solution (5mM) was prepared by adding DMSO (Carl Roth GmbH, Karlsruhe, Germany) to the CFSE vial. The stock solution was subsequently diluted 1:5000 in 1x Dulbecco's phosphate buffer solution (dPBS; Gibco life Technologies, United Kingdom) to obtain a final CFSE concentration of 6µM. Following another centrifugation step, supernatant was discarded and the appropriate volume of CFSE working solution (final concentration: 1µM) was added to the responder cells. Cells were then incubated for 20 min at 37°C in the dark.

To stop the staining reaction, RPMI supplemented (5x volume of CFSE) was added at room temperature. After short incubation of 5 min, the cells were centrifuged and resuspended in PRMI supplemented with FBS and penicillin/ streptomycin. After labeling, cells were counted using a ViCell CR cell counter (Beckman Coulter, Krefeld, Germany) and adjusted to 4 x 10^6 cells per mL in RPMI supplemented additionally containing 0.01 % β-Mercaptoethanol (Gibco®, Thermo Fisher Scientific, Waltham, MA, USA).

The Treg suppression assay was adapted from Collison and Vignali (2011).

Three conditions were plated in a round-bottom 96 well plate:

- Basal: CFSE-stained responder cells (2 x 10^5 cells per well),
- NoTreg: CFSE-stained responder cells (2 x 10^5 cells per well) + aCD3/ aCD28 (100 µL per well, pre-coated),
- Treg: CFSE-stained responder cells (2 x 10^5 cells per well) + aCD3/ aCD28 + MACS-separated $CD4^+CD25^+$ splenocytes (6 x 10^6 cells per well).

Each condition was performed with six technical replicates. Wells assigned to the NoTreg and Treg conditions were pre-incubated at least 24 h with anti-CD3 antibody (Thermo Fisher Scientific, Waltham, MA, USA). Immediately before plating responder cells and $CD4^+CD25^+$ splenocytes, wells were washed once with PBS (Gibco life Technologies, UK), followed by the addition of anti-CD28 antibody (Thermo Fisher Scientific, Waltham, MA, USA). After plating, cells were incubated for 48 h at 37 °C in an incubator with 5 % CO_2. Subsequently, plated cells were harvested by centrifugation, resuspended in FACS buffer and transferred into FACS tubes, and analyzed by flow cytometry to assess responder cell proliferation. Suppressive capacity of splenic $CD4^+CD25^+$ T cells was determined by calculating the percentage of aCD3/C28-stimulated proliferating CFSE-labeled responder cells in presence of $CD4^+CD25^+$ T cells relative to aCD3/C28-stimulated responder cells cultured in absence of $CD4^+CD25^+$ T cells. A "Treg suppressive capacity per animal" was calculated using the following formula: [(1/ mean of proliferating cells as technical replicates per group)/ percentage of $FoxP3^+$ Tregs within $CD4^+CD25^+$ cells per animal] * percentages of $CD4^+CD25^+FoxP3^+$ Tregs. This calculation was performed to account for inter-individual differences in percentages of $CD4^+CD25^+FoxP3^+$ Tregs within total splenocytes.

35. Preparation for Ultra-High-Throughput Microbial Community Analysis

In Experiment 1, microbial community analysis was performed using fecal samples collected from adult male mice prior to and after CSC exposure, as well as from the respective BBS- and MvacATCC-treated dams at mating onset, birth, and weaning. At each sampling time point, fecal samples were snap-frozen and stored at -80 °C until further processing. Microbial DNA was extracted from fecal samples using the PowerSoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. Subsequent 16S rRNA gene amplicon sequencing and bioinformatics analyses were conducted by Novogene GmbH (Munich, Germany). Briefly, Novogene's in-house protocol was applied as follows: marker genes within the isolated DNA were PCR-amplified targeting the V4 hypervariable region of the 16S rRNA gene, incorporating a unique barcode identifier for each sample. PCR reactions contained 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 2 µM of forward and reverse primers, and approximately 10 ng template DNA. Thermal cycling conditions consisted of an initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 sec, annealing at 50 °C for 30 sec, and elongation at 72 °C for 30 sec, with a final elongation step at 72 °C for 5 min. PCR products were mixed with an equal volume of 1x loading buffer containing SYBR Green and separated by electrophoresis on a 2 % agarose gel for visualization.

PCR products were pooled in equimolar ratios and purified using a Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) according to the recommendation of the manufacturer, including the addition of index sequences. Library quality was assessed using Qubit, real-time PCR for quantification, and bioanalyzer for the detection of size distribution. Quantified libraries were pooled and sequenced on an Illumina platform. One BBS dam at the time point birth failed quality control and was therefore excluded from all microbiome-related statistical analyses across all time points.

Bioinformatics analyses were conducted by Novogene according to their in-house pipelines. Raw sequencing data were demultiplexed, and sequence quality filtering was applied. Forward and reverse reads were trimmed and truncated based on quality scores, and observed amplicon sequence variants (ASVs) were identified. Downstream analyses were conducted using QIIME2. Diversity analyses were performed after rarefaction to an even sequencing depth of 34,861 reads per sample. Alpha (α-) diversity metrics, including observed ASVs, Shannon diversity index, Chao1, and Pielou's evenness, were evaluated following rarefaction. Beta (β-) diversity, reflecting differences in phylogenetic community composition between samples, was assessed using Bray-Curtis distance matrices and analyzed by permutational multivariate analysis of variance (PERMANOVA) using *adonis2* function in R (vegan package). Differential abundance analysis was performed using Analysis of Compositions of Microbiomes with Bias Correction 2 (ANCOM-BC2) implemented in R. This method accounts for the compositional nature of microbiome data and sample-specific biases using an expectation-maximization (E-M) algorithm. Holm-Bonferroni-adjusted *P*-values (q-values) were used to determine statistical significance at taxonomic level, and both the direction, as well as the magnitude of differential abundance were evaluated using estimated log fold change (LFCs). Unclassified taxa were excluded prior to ANCOM-BC2 analysis. For microbial abundance analyses, only taxa exceeding a relative abundance threshold of 0.1 % in the majority of samples were considered.

36. Mammary gland tissue homogenization and assessment of C3 mRNA expression

Fur was first removed from the mammary gland tissue using a scalpel. Subsequently, 10 mg of mammary gland tissue were added to 350 µL RLT buffer containing 1 % β-Mercaptoethanol in lysis tubes (innuSPEED Lysis Tube P, Innuscreen GmbH). Samples were homogenized using the "soft tissue 2" program of the SpeedMill homogenizer. RNA isolation and RT-qPCR were performed as previously reported (Li et al., 2024).

Prior to RNA isolation according to the manufacturer's protocol (Qiagen Qiashredder™, Hilden Germany), mammary gland tissue homogenates were centrifuged (300 g, 5 min, at 4 °C). RNA concentration was quantified using the Qubit 2.0 fluorometer and the corresponding Qubit RNA CR Assay Kit (Thermo Fisher Scientific). cDNA was synthesized using the AffinityScript qPCR cDNA Synthesis Kit (Agilent Technologies) with oligo(dT) primers and stored at -80°C for further utilization. The Brilliant III Ultra-Fast SYBR Green QPCR Master Mix in combination with the qPCR cycler Mx3000P (both from Agilent Technologies) were used to perform RT-qPCR. Primers were obtained from Biomer.net (Ulm, Germany) and Qiagen (Hilde, Germany). Relative gene expression was calculated using the $2^{-\Delta\Delta t}$ method (Schmittgen and Livak, 2008) and as fold change compared to controls. For real-time PCRs, complement C3 primers (Qiagen; Cat. Nr.: QT001109270) and self-designed HPRT1 primers (Biomers) were used, with HPRT1 serving as the housekeeping gene.

37. Statistical Analysis

Statistical analyses and graphical visualization of data were performed using GraphPad Prism (Version 10.1.1, GraphPad Software, LCC), except for microbiome data, which were analyzed separately as described in the section "Microbial Community Analysis". Data were tested for normal distribution using Kolmogorov-Smirnov test with Lilliefors' correction. For normally distributed datasets, extreme outliers within each group were identified using the Grubbs test (Grubbs, 1969) and excluded from subsequent statistical analyses. Parametric statistical tests were applied for normally distributed datasets, including two-tailed Student's *t*-test for comparisons between two independent groups (with Welch's correction applied when appropriate), two-way analysis of variance (ANOVA) for comparisons involving two or more independent groups across two factors, and the linear-mixed-model (LMM) for analyses involving repeated or dependent measurements across multiple factors. Non-parametric statistical tests were used for datasets that did not meet assumptions of normality, including Mann-Whitney *U* test (MWU) for comparisons between two independent samples, the Wilcoxon matched-pairs signed rank test for comparisons between two dependent samples, and the Friedman ANOVA for comparisons involving more than two dependent samples. Correlational analyses in non-normally distributed data were performed using Spearman correlation. For analyses involving more than two groups with significant main effects, *post hoc* analysis was conducted using Bonferroni pairwise comparisons in parametric statistics or Dunn's multiple comparison in non-parametric statistics. Data are presented as mean + standard error of the mean (SEM) with individual data points displayed as black dots representing individual animals/samples within each experimental group. The significance level was set at $P \leq 0.05$. Excluded outliers and exact *P*-values are reported in the "Results" section of this thesis.

Results

Intergenerational effects of *M. vaccae* ATCC 15483^T on the negative behavioral, physiological and immunological consequences of chronic psychosocial stress in adult C57BL/6N male and female offspring

To investigate whether repeated administrations (i.g.) of *M. vaccae ATCC* 15483^T to nulliparous female mice prior to mating protect the offspring from negative behavioral consequences of CSC or SIP, male and female offspring were tested for social and anxiety-related behaviors one day prior to and at the end of the CAS exposure using the SPAT and the OF/NO, respectively (**Figure 1**) (Schiele et al., 2026).

In detail, *M. vaccae* ATCC 15483^T did not affect social preference in both male (**Figure 1a**) and female (**Figure 1b**) offspring prior to CAS exposure. Neither stress nor treatment affected male offspring during the open field exploration (**Figure 1c**, Distance moved; **Figure 1d**, Entries into inner zone; **Figure 1e**, Time in inner zone). However, maternal treatment with *M. vaccae* ATCC 15483^T (i.g.) intergenerationally prevented mild signs of CSC-induced general anxiety-related behavior in male offspring. This was indicated by a significant reduction in the number of entries into to the contact zone during the novel object condition in CSC males of BBS-treated dams compared to SHC males of BBS-treated dams (**Figure 1f**; MWU: $P = 0.034$), an effect that was absent in CSC mice of MvacATCC-treated mothers (**Figure 1f**; MWU: $P = 0.002$). Additionally, CSC males of MvacATCC-treated mothers spent significantly more time in the contact zone during the novel object exploration compared with CSC mice of BBS-treated dams (**Figure 1g**; MWU: $P < 0.001$).

Furthermore, females of BBS-treated dams exposed to SIP traveled significantly more than their respective GHC controls (**Figure 1h**; MWU: $P = 0.045$), while this effect was not found in SIP-exposed females of MvacATCC-treated dams. In the open field condition, the parameter entries into inner zone was not affected (**Figure 1i**). However, time spent in inner zone tended to be reduced in SIP-females of M. vaccae ATCC 15483^T-treated dams compared with their respective GHC controls (**Figure 1j**; MWU: P = 0.055). During the novel object exploration, no significant differences were found in the parameters entries into the contact zone or time in contact zone in female offspring (**Figure 1k, l**).

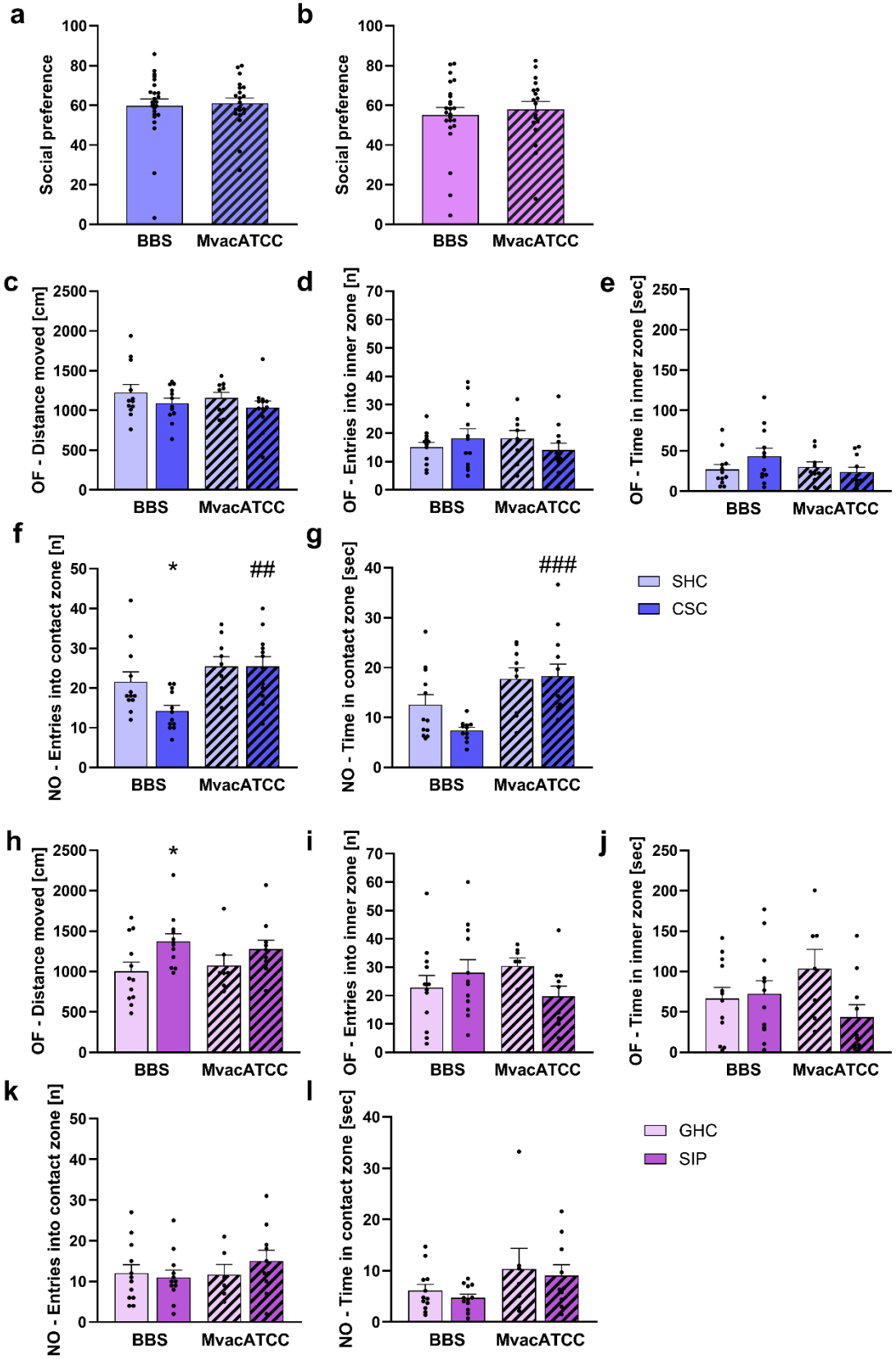


Figure 1 | Intergenerational effects of repeated i.g. administrations of *Mycobacterium vaccae* ATCC 15483^T to nulliparous female C57BL/6N mice prior to mating on social and general anxiety-related behavior of male and female offspring before and after exposure to chronic psychosocial stress during adulthood (Experiment 1).

The social preference/avoidance test was performed prior to chronic adult stressor exposure in experimental male **(a)** and female **(b)** offspring. **a, b)** Social preference (time spent in the direct contact zone (during social cage exposure) divided by total time spent in direct contact zone (during empty + social cage exposure)). The open field (OF)/novel object (NO) test was performed at the end of chronic adult stress exposure in experimental male **(c-g)** and female **(h-l)** offspring. **c, h)** Distance moved during OF exposure. **d, i)** Entries into the inner zone during OF exposure. **e, j)** Time in inner zone during OF exposure. **f, k)** Entries into contact zone during NO exposure. **g, l)** Time in the contact zone during NO exposure. Data are presented as mean + SEM including individual values (dots). Blue bars: male offspring (light blue: single-housed controls (SHC), dark blue: chronic subordinate colony housing (CSC)), purple bars: female offspring (light purple: group-housed controls (GHC), dark purple: social instability paradigm (SIP)). Non-hatched bars: borate-buffered saline (BBS), hatched bars: M. vaccae ATCC 15483^T (MvacATCC); * $P \leq 0.05$ versus (vs.) respective SHC or GHC condition, ## $P \leq 0.01$, ### $P \leq 0.001$ vs. respective BBS condition. Social preference/avoidance test: N = 15 – 24 per group; open-field/novel object test: $N = 7 – 12$ per group. In case individual samples had to be excluded due to methodological reasons, this is reported in the respective materials and methods section 8. Adapted from Schiele et al. (2026), licensed under CC BY 4.0.

To assess whether *M. vaccae* ATCC 15483^T intergenerationally protected male and female offspring from the negative physiological consequences of chronic psychosocial stress, well-established stress-sensitive organs, including the adrenal glands, thymus, spleen and long bones such as tibia, were investigated for typical CSC- or SIP-related effects (**Figure 2**) (Schiele et al., 2026).

The data indicated that CSC induced a significant increase in relative adrenal weight in both experimental male groups of BBS- and MvacATCC-treated dams (**Figure 2a**; 2WayANOVA: $F(CAS)_{1,41} = 83.66$, $P < 0.001$) compared with their respective SHC group (Bonferroni: BBS-SHC: $P < 0.001$; MvacATCC-SHC: $P < 0.001$). However, the CSC-exposed male offspring of MvacATCC-treated dams showed a significant lower adrenal weight than CSC-exposed male offspring of BBS-treated dams (**Figure 2a**; 2WayANOVA: $F(Treatment)_{1,41} = 9.779$; $P = 0.003$; Bonferroni: vs. BBS-CSC: $P < 0.001$). Moreover, a significant interaction effect between CAS and maternal *M. vaccae* ATCC 15483^T treatment was revealed (**Figure 2a**; 2WayANOVA: $F(CAS \times Treatment)_{1,41} = 6.652$; $P = 0.014$). In addition, CSC significantly decreased the relative thymus weight in male mice born to BBS-mothers (**Figure 2b**; 2WayANOVA: $F(CAS)_{1,41} = 11.59$; $P = 0.001$, Bonferroni: $P < 0.001$), an effect that was absent in male mice born to MvacATCC-mothers (**Figure 2b**; 2WayANOVA: $F(Treatment)_{1,41} = 19.78$; $P < 0.001$, Bonferroni: $P < 0.001$). A significant interaction between CAS and maternal *M. vaccae* ATCC 15483^T treatment was also found (**Figure 2b**; 2WayANOVA: $F(CAS \times Treatment)_{1,41} = 13.19$; $P < 0.001$).

Tibia length was significantly reduced by CSC exposure in males of BBS-treated dams (**Figure 2c**; MWU: $P = 0.010$). In contrast, CSC-exposed males of MvacATCC-treated dams had significantly longer tibiae than CSC males of BBS-treated dams (**Figure 2c**; MWU: $P =$

0.005). Independent of maternal treatment, relative spleen weight was significantly increased in CSC mice in both the BBS (**Figure 2d**; MWU: $P < 0.001$) and the MvacATCC (**Figure 2d**; MWU: $P < 0.001$) groups. However, a significant difference was detected between CSC-exposed male offspring of BBS- and MvacATCC-treated mothers (**Figure 2d**; MWU: $P = 0.033$). Furthermore, statistical comparison of bite wound severity between CSC-exposed males born to BBS-treated dams (Mean + standard error of mean (SEM): 47.92 + 11.00) and CSC-exposed males born to MvacATCC-treated dams (Mean + SEM: 35.00 + 5.938) revealed no significant group differences (**Figure 2e**).

In female mice, neither SIP nor maternal *M. vaccae* ATCC 15483^T treatment affected any of the assessed readouts (**Figure 2f-i**). Of note, bite wounds were not assessed in females.

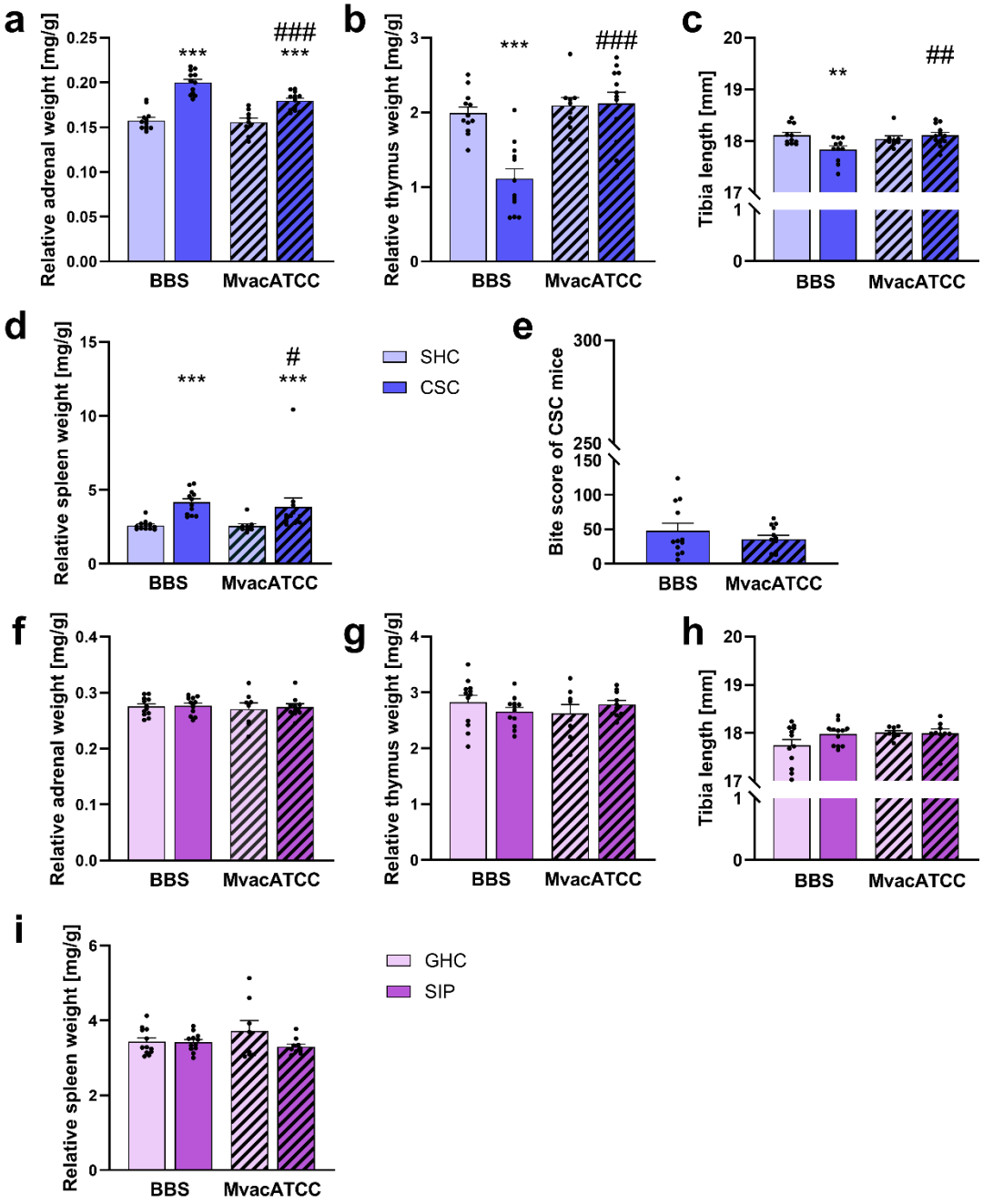


Figure 2 | Intergenerational effects of repeated administrations (i.g.) of *Mycobacterium vaccae* ATCC 15483^T to nulliparous female C57BL/6N mice prior to mating on the negative physiological consequences of chronic psychosocial stress during adulthood in male and female offspring (Experiment 1).
a-i) show data from experimental male **(a-e)** and female **(f-i)** offspring exposed to either chronic adult stress (males: chronic subordinate colony housing, CSC; females: social instability paradigm, SIP) or no-stress (males: single-housed controls, SHCs; females: group-housed controls, GHC) during adulthood. Relative **a, f)** adrenal and **b, g)** thymus weight. **c, h)** Tibia length. **d, i)** Relative spleen weight. **e)** Bite score in CSC-exposed male offspring. Data are presented as mean + SEM including individual values (dots). Blue bars: male offspring, Purple bars: female offspring. Non-hatched bars: borate-buffered (BBS), hatched bars: M. vaccae ATCC 15483^T (MvacATCC); ** $P \leq 0.01$, and *** $P \leq 0.001$ vs. respective SHC condition, # $P \leq 0.05$, ## $P \leq 0.01$, and ### $P \leq 0.001$ vs. respective BBS condition. $N = 8 – 12$ per group. In case individual samples had to be excluded due to methodological reasons, this is reported in the respective materials and methods sections 17 and 20. Adapted from Schiele et al. (2026), licensed under CC BY 4.0.

Moreover, the effect of repeated i.g. *M. vaccae* ATCC 15483^T administrations on immunological parameters affected by CAS exposure in male (**Figure 3**) and female (**Figure 4**) offspring was assessed (Schiele et al., 2026).

As shown above, CSC exposure is accompanied by bite injuries inflicted by the resident male in the experimental C57BL/6N mice and is therefore associated with splenic enlargement. Next, splenocytes were isolated *in vitro* and further processed using various methods. Western blot analysis revealed that CSC exposure significantly increased the splenic TLR2 (**Figure 3a**; 2WayANOVA, 1 outlier in MvacATCC-SHC group: $F(CAS)_{1,40} = 7.449$; $P = 0.009$, Bonferroni: $P = 0.016$) and TLR4 (**Figure 3b**; MWU: $P = 0.001$) protein levels in male offspring of BBS-treated dams compared to their respective SHC males. This effect was ameliorated (TLR2, **Figure 3a**) or prevented (TLR4, **Figure 3b**; MWU: $P = 0.024$) in CSC-exposed males of MvacATCC-treated dams compared to the BBS-CSC group.

Moreover, CSC offspring of BBS-treated dams showed significantly increased basal and LPS-induced ex vivo cell viability of isolated splenocytes in absence of corticosterone (CORT 0 µM) compared to respective SHC offspring (**Figure 3c**; basal – MWU: $P = 0.001$, LPS – MWU: $P < 0.001$). This effect was not found in CSC offspring of *M. vaccae* ATCC 15483^T-treated dams (**Figure 3c**; basal – MWU: $P = 0.001$). In contrast, SHC males of MvacATCC-treated mothers displayed significantly higher *ex vivo* splenic cell viability under LPS-stimulated conditions (**Figure 3c**; LPS – MWU: $P = 0.001$) compared to SHC males from BBS-treated mothers. In general, LPS stimulation significantly increased splenocyte viability in the absence of CORT (0 µM) compared with the basal condition (**Figure 3c**; Wilcoxon: BBS-SHC: $P < 0.001$, BBS-CSC: $P < 0.001$, BBS-MvacATCC: $P = 0.004$, MvacATCC-CSC: $P = 0.002$). Similarly, delta cell viability (LPS values minus basal values) was significantly increased by CSC in males of BBS-treated dams compared with their respective SHC males (**Figure 3d**; MWU: $P < 0.001$).

In contrast, maternal *M. vaccae* ATCC 15483^T treatment increased delta cell viability in SHC mice (**Figure 3d**; MWU: $P = 0.006$) compared to SHC mice born to BBS-treated dams in the absence of CORT (0 µM). Additionally, when compared with the 0 µM CORT condition (set to 100 %), delta *ex vivo* cell viability in CSC offspring of MvacATCC-treated dams was not significantly reduced in the presence of 0.1 µM CORT (**Figure 3e**), while all other groups showed significantly reduced delta *ex vivo* cell viability under the 0.1 µM condition (**Figure 3e**; Friedman: BBS-SHC: χ^2 (2, 11) = 11.09, $P = 0.003$, Dunn's: $P = 0.017$; MvacATCC-SHC: χ^2 (2, 9) = 14.00, $P < 0.001$, Dunn's: $P = 0.014$; MvacATCC-CSC: χ^2 (2, 10) = 15.20, $P < 0.001$, Dunn's: $P = 0.014$).

A similar pattern was found in all experimental male groups when comparing the delta *ex vivo* cell viability in the 5.0 µM CORT condition with the 0 µM CORT condition set to 100 % (**Figure 3e**; Friedman: BBS-SHC: χ^2 (2, 11) = 11.09, P = 0.003, Dunn's: P = 0.009; BBS-CSC: χ^2 (2, 12) = 16.67, P < 0.001, Dunn's: P < 0.001; MvacATCC-SHC: χ^2 (2, 9) = 14.00, P < 0.001, Dunn's: P = 0.001; MvacATCC-CSC: χ^2 (2, 10) = 15.20, P < 0.001, Dunn's: P = 0.005). However, intergenerational *M. vaccae* ATCC 15483^T treatment significantly reduced delta *ex vivo* cell viability in both SHC (Figure 3e; MWU: P = 0.046) and CSC males (**Figure 3e**; MWU: P = 0.003) under the 0.1 µM CORT condition, and in the CSC group under the 5 µM CORT condition (**Figure 3e**; MWU: P = 0.021), compared with the respective experimental group of BBS-treated mothers.

Furthermore, FACS analysis revealed that CSC significantly reduced splenic $CD4^+CD25^+FoxP3^+$ Treg numbers (in percent (%) of all splenocytes) in male offspring of BBS-treated mothers when compared to the respective SHC male offspring (**Figure 3f**; 2WayANOVA: $F(CAS)_{1,41}$ = 15.58; P < 0.001, $F(Treatment)_{1,41}$ = 4.627; P = 0.037, $F(CAS*Treatment)_{1,41}$ = 10.58; P = 0.002, Bonferroni: P < 0.001). This effect was intergenerationally prevented in CSC-exposed males by maternal *M. vaccae* ATCC 15483^T treatment (**Figure 3f**; 2WayANOVA: $F(CAS)_{1,41}$ = 15.58; P < 0.001, $F(Treatment)_{1,41}$ = 4.627; P = 0.037, $F(CAS*Treatment)_{1,41}$ = 10.58; P = 0.002, Bonferroni: P < 0.001). In contrast, maternal i.g. *M. vaccae* ATCC 15483^T administration significantly increased splenic $CD4^+CD25^+FoxP3^+$ Treg counts (in % of $CD4^+CD25^+$ splenocytes) in male offspring exposed to CSC in comparison to the BBS-CSC group (**Figure 3g**; 2WayANOVA: $F(Treatment)_{1,41}$ = 8.068; P = 0.007, Bonferroni: P = 0.014).

To determine whether the suppressive functionality of $CD4^+CD25^+$ Tregs was also affected by CSC exposure and intergenerationally protected by *M. vaccae* ATCC 15483^T, a Treg suppression assay was performed. After 48 h of incubation, the anti(a)-CD3/28 stimulation induced proliferation of CFSE-labeled (-stained) responder cells compared with the basal condition (**Figure 3h**; Wilcoxon: P = 0.031). The aCD3/28 condition was set to 100 % and proliferation of CFSE-stained responder cells was analyzed after 48 h following the addition of $CD4^+CD25^+$ activated T cells of each experimental group (Figure 3i). In comparison to the aCD3/28 condition, both no-stressed and stressed male groups of BBS-treated mothers (**Figure 3i**; Wilcoxon: SHC: P = 0.031, CSC: P = 0.031), as well as SHC males of MvacATCC-treated mothers (**Figure 3i**; Wilcoxon: P = 0.031) showed significantly reduced proliferation of CFSE-stained cells.

Additionally, maternal *M. vaccae* ATCC 15483^T treatment significantly increased the numbers of proliferated CFSE-stained responder cells in both SHC (**Figure 3i**; MWU: $P = 0.004$) and CSC males (**Figure 3i**; MWU: $P = 0.002$) compared with the respective BBS control groups.

The "Treg suppression capacity per animal" was calculated as described in the Material and Methods (Section 34). Again, offspring of dams treated with *M. vaccae* ATCC 15483^T were protected (**Figure 3j**; 2WayANOVA: $F(CAS*Treatment)_{1,41} = 13.55$; $P = 0.001$, $F(CAS)_{1,41} = 34.36$; $P = 0.001$, Bonferroni: $P = 0.027$) against the CSC-induced reduction in Treg suppressive capacity (**Figure 3j**; 2WayANOVA: $F(CAS*Treatment)_{1,41} = 13.55$; $P = 0.001$, $F(CAS)_{1,41} = 34.36$; $P = 0.001$, Bonferroni: $P = 0.001$). Furthermore, the calculated Treg suppression capacity per animal was significantly reduced in SHC males of MvacATCC-treated dams (**Figure 3j**; 2WayANOVA: $F(CAS*Treatment)_{1,41} = 13.55$; $P = 0.001$, $F(CAS)_{1,41} = 34.36$; $P = 0.001$, Bonferroni: $P = 0.024$) compared to the respective controls of BBS-treated dams.

The percentages of splenic myeloid cell subpopulations were assessed using FACS analysis. CSC exposure significantly (or by trend) increased the percentages of monocytes/ MO-MDSCs (**Figure 3k**; MWU: BBS: $P < 0.001$, MvacATCC: $P = 0.001$), neutrophils (**Figure 3l**, MWU: BBS: $P = 0.007$, MvacATCC: $P = 0.060$), and PMN-MDSCs (**Figure 3m**, MWU: BBS: $P < 0.001$, MvacATCC: $P < 0.001$) independent of maternal BBS or *M. vaccae* ATCC 15483^T treatment. In SHC males of MvacATCC-treated dams, percentages of monocytes/ MO-MDSCs were increased in comparison to the respective control males of BBS-treated dams (**Figure 3k**; MWU: $P = 0.043$).

Finally, the chemotactic activity of isolated white blood cells was significantly increased by CSC exposure in both groups of BBS- (**Figure 3n**; MWU: $P = 0.002$) and MvacATCC-treated mothers (**Figure 3n**; MWU: $P = 0.002$), which was even more pronounced following maternal *M. vaccae* ATCC 15483^T treatment (**Figure 3n**; MWU: $P = 0.002$).

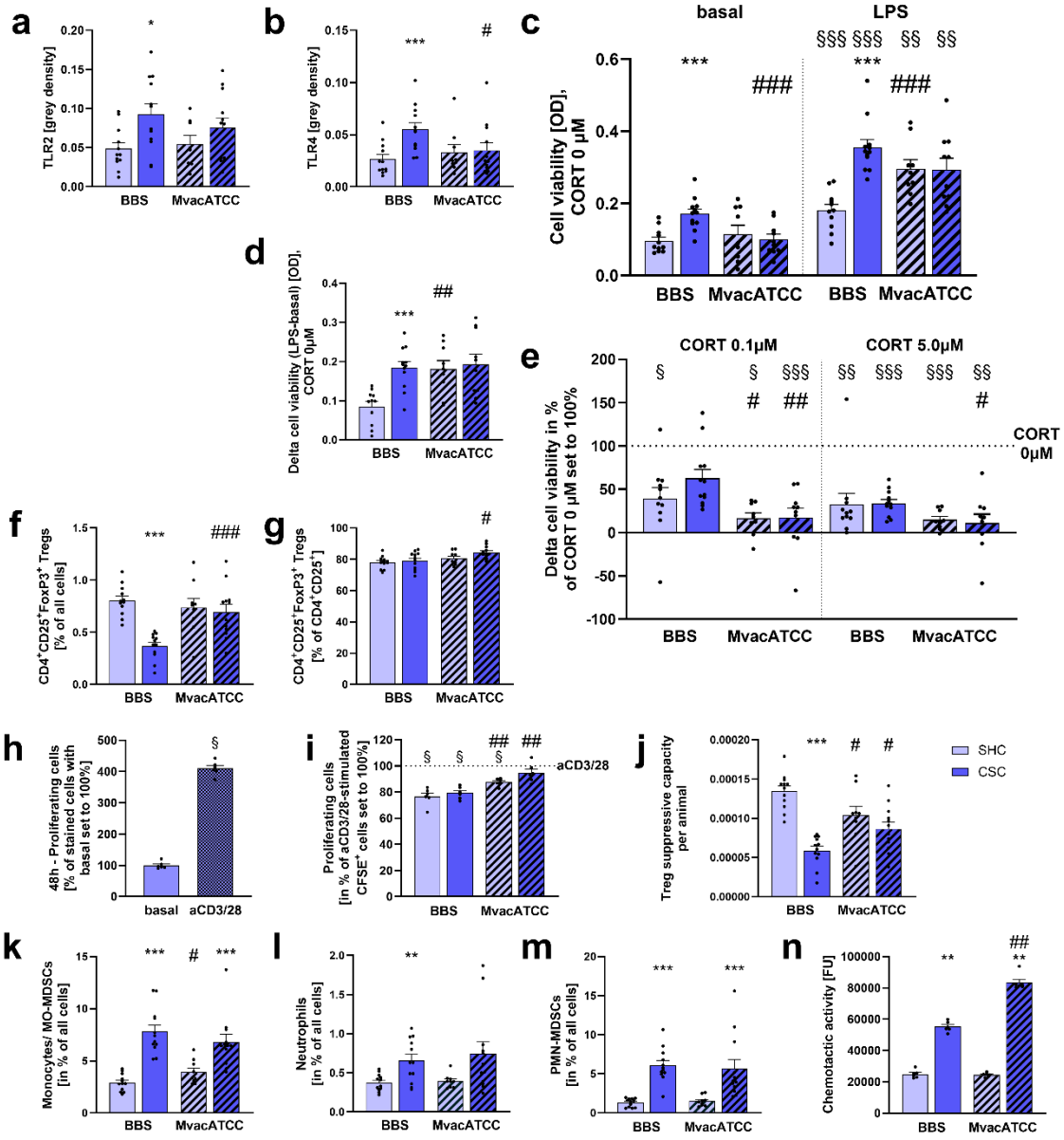


Figure 3 | Intergenerational effects of repeated administrations (i.g.) of *Mycobacterium vaccae* ATCC 15483^{T} to nulliparous female C57BL/6N mice prior to mating on the negative immunological consequences of chronic psychosocial stress during adulthood in male offspring (Experiment 1).
Splenic protein expression of **a)** toll-like receptor (TLR)2 and **b)** TLR4. **c)** *Ex vivo* cell viability under basal and lipopolysaccharide (LPS) conditions at 0 µM corticosterone (CORT). **d)** Delta (LPS minus basal) *ex vivo* cell viability at 0 µM CORT. **e)** Delta *ex vivo* cell viability at 0.1 µM and 5 µM CORT in percent (%) of 0 µM CORT set to 100 %. **f)** Splenic $CD4^{+}CD25^{+}FoxP3^{+}$ Tregs (in percent (%) of all splenocytes), **g)** splenic $CD4^{+}CD25^{+}FoxP3^{+}$ Tregs (in % of $CD4^{+}CD25^{+}$ cells). **h)** *Ex vivo* proliferation of CFSE-stained responder splenocytes from naïve male mice after exposure to 48 h basal and anti(a)CD3/28-stimulated condition. Cells were not co-cultured with $CD4^{+}CD25^{+}$ T cells from experimental male mice. **i)** Proliferation of CFSE-stained responder splenocytes from naïve male mice (in % of aCD3/28-stimulated $CFSE^{+}$ cells set to 100 %) co-cultured with $CD4^{+}CD25^{+}$ T cells from experimental male mice. **j)** Calculated Treg suppressive capacity per male experimental mouse. Percentage of splenic **k)** $CD11b^{+}Ly6G^{-}Ly6C^{+}$ monocytes/ monocyte-like (MO) myeloid-derived suppressor cells (MDSCs), **l)** $CD11b^{+}Ly6G^{+}Ly6C^{-}$ neutrophils, **m)** $CD11b^{+}Ly6G^{+}Ly6C^{+}$ polymorphonuclear (PMN)-MDSCs. **n)** Chemotactic activity of isolated neutrophils ($N = 6$ technical replicates per group). Data are presented as mean + SEM including individual values (dots). Light blue bars: single-housed controls (SHC), dark blue bars: chronic subordinate colony (CSC) housing.

Non-hatched bars: borate-buffered saline (BBS), hatched bars: *M. vaccae* ATCC 15483^T (MvacATCC). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ vs. respective SHC condition; $^{\#}$ $P \leq 0.05$, $^{\#\#}$ $P \leq 0.01$, $^{\#\#\#}$ $P \leq 0.001$ vs. respective BBS condition; § $P \leq 0.05$; §§ $P \leq 0.01$; §§§ $P \leq 0.001$ vs. respective basal or 0 µM CORT (set to 100 %) condition. $N = 8 – 12$ per group. Adapted from Schiele et al. (2026), licensed under CC BY 4.0.

In female offspring, neither SIP nor *M. vaccae* ATCC 15483^T affected most of the assessed immunological parameters (**Figure 4a-e, g-j, l-n**), with only two parameters significantly altered (Schiele et al., 2026).

First, maternal *M. vaccae* ATCC 15483^T treatment significantly increased the counts of $CD4^+CD25^+FoxP3^+$ Tregs (in % of all splenocytes) in females exposed to SIP (**Figure 4f**; 2WayANOVA: $F(Treatment)_{1,38} = 7.455$; $P = 0.010$, Bonferroni: $P = 0.029$) compared to respective GHC females. Second, SIP induced a significant increase in the percentage of monocyte/MO-MDSCs (in % of all cells), independent of the factor treatment (**Figure 4k**; 2WayANOVA, 3 outliers in the following groups: BBS-GHC, MvacATCC-GHC, MvacATCC-SIP: $F(CAS)_{1,35} = 14.76$; $P < 0.001$, Bonferroni: BBS: $P = 0.010$, MvacATCC: $P = 0.034$).

Furthermore, the splenic GC sensitivity assay functioned as expected, as a significant increase in *ex vivo* cell viability was found in all experimental female groups under LPS stimulation (**Figure 4c**; Wilcoxon: BBS-GHC and BBS-SIP: $P < 0.001$, MvacATCC-GHC: $P = 0.008$ and MvacATCC-SIP: $P = 0.002$) vs. basal condition. Additionally, GC sensitivity of isolated splenocytes was evident in all experimental female groups under both the 0.1 µM CORT condition (**Figure 4e**; Friedman: BBS-GHC: $\chi2$ (2, 11) = 16.91, $P < 0.001$, Dunn's: $P = 0.004$; BBS-SIP: $\chi2$ (2, 12) = 18.67, $P < 0.001$, Dunn's: $P = 0.003$; MvacATCC-GHC: $\chi2$ (2, 8) = 10.90, $P = 0.002$, Dunn's: $P = 0.018$; MvacATCC-SIP: $\chi2$ (2, 9) = 16.22, $P < 0.001$, Dunn's: $P = 0.055$) and the 5.0 µM CORT condition (**Figure 4e**; Friedman: BBS-GHC: $\chi2$ (2, 11) = 16.91, $P < 0.001$, Dunn's: $P < 0.001$; BBS-SIP: $\chi2$ (2, 12) = 18.67, $P < 0.001$, Dunn's: $P < 0.001$; MvacATCC-GHC: $\chi2$ (2, 8) = 10.90, $P = 0.002$, Dunn's: $P = 0.012$; MvacATCC-SIP: $\chi2$ (2, 9) = 16.22, $P < 0.001$, Dunn's: $P < 0.001$).

Lastly, aCD3/28 stimulation induced proliferation of CFSE-stained responder cells compared with the basal condition after 48 h of incubation (**Figure 4h**; Wilcoxon: $P = 0.031$).

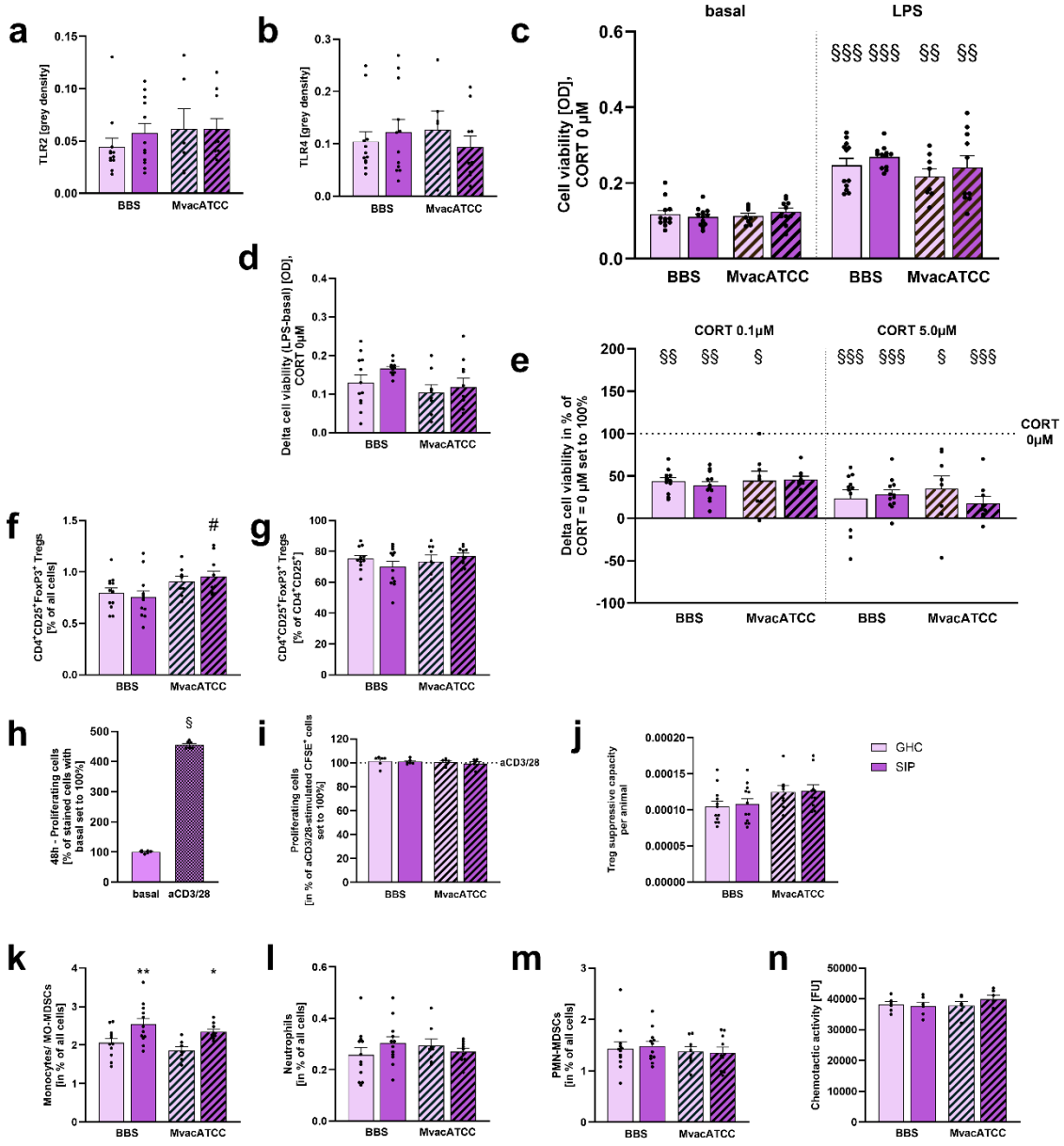


Figure 4 | Intergenerational effects of repeated administrations (i.g.) of Mycobacterium vaccae ATCC 15483T to nulliparous female C57BL/6N mice prior to mating on the negative immunological consequences of chronic psychosocial stress during adulthood in male offspring (Experiment 1).
Splenic protein expression of **a)** toll-like receptor (TLR)2 and **b)** TLR4. **c)** Ex vivo cell viability under basal and lipopolysaccharide (LPS) conditions at 0 µM corticosterone (CORT). **d)** Delta (LPS minus basal) ex vivo cell viability at 0 µM CORT. **e)** Delta ex vivo cell viability at 0.1 µM and 5 µM CORT in percent (%) of 0 µM CORT set to 100 %. **f)** Splenic $CD4^+CD25^+FoxP3^+$ Tregs (in percent (%) of all splenocytes), **g)** splenic $CD4^+CD25^+FoxP3^+$ Tregs (in % of $CD4^+CD25^+$ cells). **h)** Ex vivo proliferation of CFSE-stained responder splenocytes from naïve female mice after exposure to 48 h basal and anti(a)CD3/28-stimulated condition. Cells were not co-cultured with $CD4^+CD25^+$ T cells from experimental female mice. **i)** Proliferation of CFSE-stained responder splenocytes from naïve female mice (in % of aCD3/28-stimulated CFSE+ cells set to 100 %) co-cultured with $CD4^+CD25^+$ T cells from experimental female mice. **j)** Calculated Treg suppressive capacity per female experimental mouse. Percentage of splenic **k)** $CD11b^+Ly6G^-Ly6C^+$monocytes/ monocyte-like (MO) myeloid-derived suppressor cells (MDSCs), **l)** $CD11b^+Ly6G^+Ly6C^-$ neutrophils, **m)** $CD11b^+Ly6G^+Ly6C^+$ polymorphonuclear (PMN)-MDSCs. **n)** Chemotactic activity of isolated neutrophils (N = 6 technical replicates per group). Data are presented as mean + SEM including individual values (dots). Light purple bars: group-housed controls (GHC), dark purple bars: social instability paradigm (SIP).

Non-hatched bars: borate-buffered saline (BBS), hatched bars: *M. vaccae* ATCC 15483^T (MvacATCC). * $P \leq 0.05$, ** $P \leq 0.01$ vs. respective SHC condition; # $P \leq 0.05$ vs. respective BBS condition; § $P \leq 0.05$; §§ $P \leq 0.01$; §§§ $P \leq 0.001$ vs. respective basal or 0 µM CORT (set to 100 %) condition. $N = 8 – 12$ per group. In case individual samples had to be excluded due to methodological reasons, this is reported in the materials and methods sections 26 and 29. Adapted from Schiele et al. (2026), licensed under CC BY 4.0.

M. vaccae ATCC 15483^T intergenerationally diversifies the microbiome of male offspring and increases the relative abundance of potent SCFA-producing taxa

To determine possible mechanisms underlying the intergenerational chronic stress-protective effects of *M. vaccae* ATCC 15483^T in male offspring, the fecal microbiome composition was assessed prior to (**Figure 5a-f**) and after (**Figure 5g-l**) CSC exposure (Schiele et al., 2026).

Permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis matrix of the fecal microbiome revealed significant differences in β-diversity between males of MvacATCC- and BBS-treated dams before CSC exposure (**Figure 5a**, PERMANOVA; Bray-Curtis; Pseudo-$F = 6.4497$; $P = 0.001$). Moreover, α-diversity metrics reflecting richness and evenness of the bacterial microbiome prior to CSC (**Figure 5b-e**) were analyzed. The number of observed ASVs (**Figure 5b**, T-Test with Welch's correction: $P < 0.001$), Shannon diversity index (**Figure 5c**, T-Test: $P = 0.001$), and Chao1 index (**Figure 5d**, T-Test: $P < 0.001$) were significantly increased in male offspring of MvacATCC-treated dams compared with males born to BBS-treated dams. In contrast, Pielou's evenness was not intergenerationally affected by maternal *M. vaccae* ATCC 15483^T treatment (**Figure 5e**). Spearman correlation analysis between α-diversity metrics (observed ASVs, Shannon and Chao1 index) before CSC exposure and physiological as well as immunological parameters assessed after CSC revealed that the number of observed ASVs and Chao1 index correlated significantly or by trend ($P \leq 0.1$) negatively (marked in orange; exact rho and *P*-values are shown in **Figure 5f**) with parameters increased by CSC in the BBS group. These included relative adrenal and spleen weight, splenic TLR4 protein levels, splenic monocytes/ MO-MDSCs and PMN-MDSC counts, basal splenic *ex vivo* cell viability in the absence of CORT and delta (LPS-basal) splenic *ex vivo* cell viability in the presence of 0.1 µM and 5 µM CORT. Conversely, positive correlations (marked in green; exact rho and *P*-values are shown in **Figure 5f**) were observed in the majority of parameters decreased by CSC in the BBS group, including relative thymus weight, $CD4^+CD25^+FoxP3^+$ Treg counts (in % of all cells), and the calculated "Treg suppressive capacity per animal".

To determine whether the above described alterations in fecal microbial composition before CSC were stress-resistant, the same microbial parameters were assessed after CSC exposure in male offspring born to MvacATCC-treated mothers and to BBS-treated mothers (Schiele et al., 2026). Again, PERMANOVA on Bray-Curtis matrix revealed significant differences in β-diversity between males of MvacATCC- and BBS-treated dams after CSC exposure (**Figure 5g**, PERMANOVA; Bray-Curtis; Pseudo-F = 2.5185; P = 0.003). Furthermore, the same α-diversity metrics reflecting richness, including number of observed ASVs (**Figure 5h**, T-Test: $P < 0.001$), Shannon index (**Figure 5i**, T-Test: $P = 0.045$), and Chao1 index (**Figure 5j**, T-Test: $P < 0.001$), were significantly higher in CSC males of dams treated with *M. vaccae* ATCC 15483^T compared with CSC males born to BBS-treated dams after CSC exposure.

In contrast, Pielou's evenness did not differ between the two CSC groups (**Figure 5k**).

Spearman correlation analysis between α-diversity metrics (observed ASVs, Shannon and Chao1 index) and physiological as well as immunological parameters assessed after CSC exposure revealed that the number of observed ASVs and/or the Chao1 index correlated significantly or by trend ($P \leq 0.1$) negatively (marked in orange; exact rho and P-values are shown in **Figure 5l**) with parameters increased by CSC in the BBS group. These included relative adrenal weight, splenic TLR2 and TLR4 protein levels, basal splenic *ex vivo* cell viability in absence of CORT and delta (LPS-basal) splenic *ex vivo* cell viability in presence of 0.1 µM CORT. Positive correlations (marked in green; exact rho and P-values are shown in **Figure 5l**) were observed in parameters decreased by CSC in the BBS group, including relative thymus weight, tibia length, $CD4^+CD25^+FoxP3^+$ Treg counts (in % of all cells and in % of $CD4^+CD25^+$ cells), and the calculated "Treg suppressive capacity per animal".

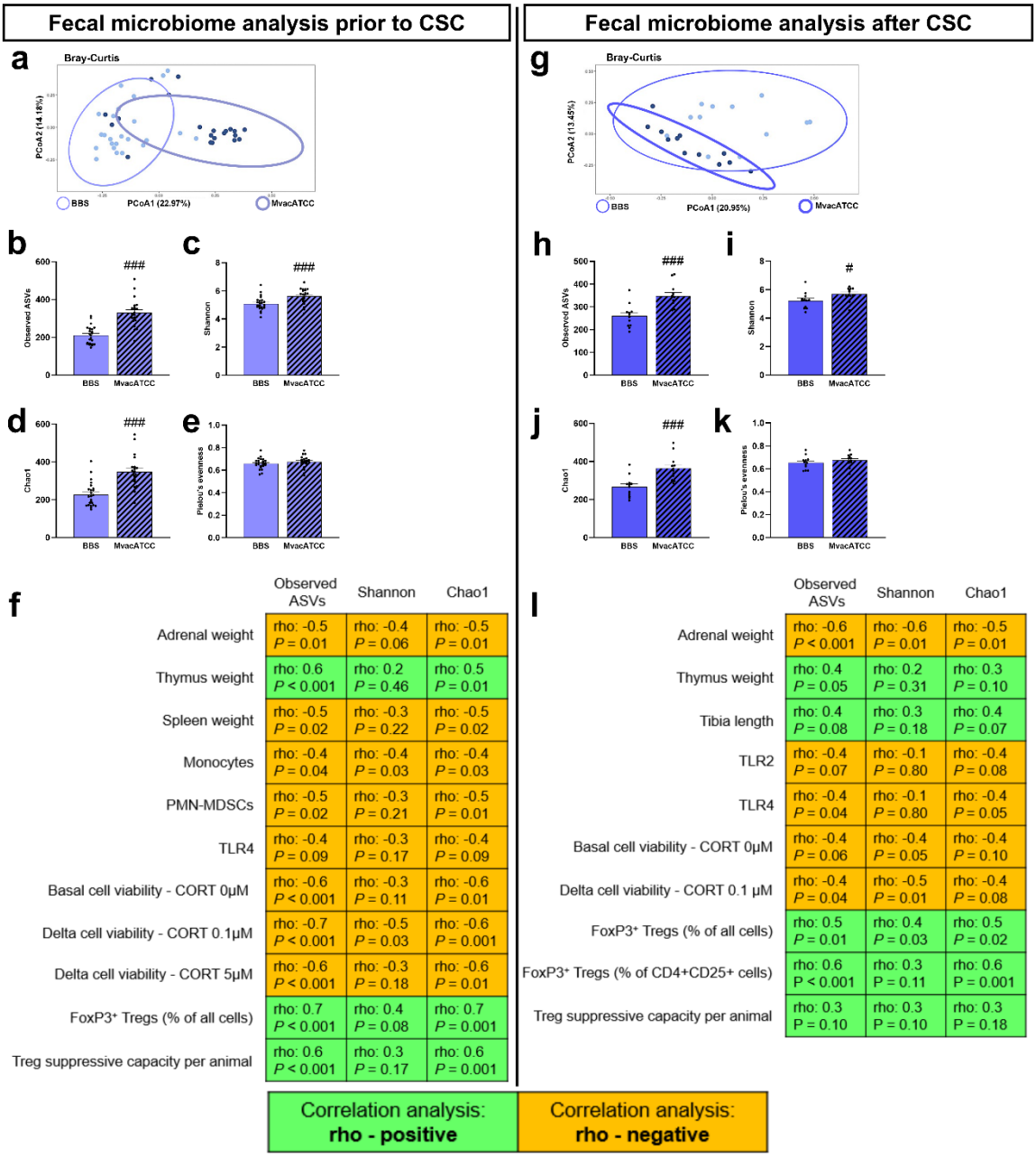


Figure 5 | Intergenerational effects of repeated i.g. administration of *M. vaccae* ATCC 15483^T to nulliparous female C57BL/6N mice prior to mating on the diversity of the fecal microbiome in male offspring prior and after the exposure of chronic psychosocial stress in adulthood (Experiment 1).
Fecal microbiome analysis in male offspring prior **(a-f)** and after **(g-l)** chronic psychosocial stress exposure**. a, g)** β-diversity (Bray-Curtis) principal coordinates analysis (PCoA). **b-e, h-k)** α-diversity metrics **(b, h)** observed amplicon sequence variants (ASVs), **(c, i)** Shannon diversity index, **(d, j)** Chao1 richness estimate (Chao1) and **(e, k)** Pielou's evenness. **f, l)** Correlation analysis of α-diversity metrics with physiological and immunological parameters. (Abbreviations: polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), toll-like receptor (TLR), corticosterone (CORT), regulatory T cells (Tregs)). Data are presented as mean + SEM including individual values (dots). Non-hatched bars: borate-buffered saline (BBS), hatched bars: *M. vaccae* ATCC 15483^T (MvacATCC). # $P \leq 0.05$, ### $P \leq 0.001$ vs. respective BBS condition. $N = 8 – 24$ animals per group. Adapted from Schiele et al. (2026), licensed under CC BY 4.0.

Alterations in the community composition of the fecal microbiome in male offspring induced by intergenerational *M. vaccae* ATCC 15483^{T} administration were analyzed prior to (**Figure 6a, b**) and after (**Figure 6c, d**) CSC exposure using analysis of composition of microbiomes with bias correction (ANCOM-BC), including estimates of log fold change (LFC) and false discovery rate (FDR) (Schiele et al., 2026).

Prior to CSC exposure, ANCOM-BC revealed that the relative abundance of Desulfobacterota (phylum; LFC: 1.712, *P* (FDR corrected) < 0.001)/ Desulfovibrionales (order; LFC: 1.712, *P* (FDR corrected) = 0.001)/ Desulfovibrionaceae (family; LFC: 1.712, *P* (FDR corrected) = 0.003), Oscillospirales (order; LFC: 1.808, *P* (FDR corrected) < 0.001), Clostridia vadin BB60 group (order; LFC: 1.272, *P* (FDR corrected) = 0.010), and *Roseburia* (genus; LFC: 1.480, *P* (FDR corrected) = 0.036) were significantly increased in CSC-exposed male offspring of MvacATCC-treated dams compared with CSC-exposed males of BBS-treated dams (**Figure 6a**). In contrast, ANCOM-BC revealed that the relative abundance of Bacteroidota (phylum; LFC: -0.703, *P* (FDR corrected) = 0.002)/ Bacteroidales (order; LFC: -0.703, *P* (FDR corrected) = 0.007)/ Bacteroidaceae (family; LFC: -0.955, *P* (FDR corrected) = 0.048), Lactobacillales (order; LFC: -1.825, *P* (FDR corrected) = 0.012)/ Lactobacillaceae (family; LFC: -1.825, *P* (FDR corrected) = 0.020)/ *Lactobacillus* (genus; LFC: -2.239, *P* (FDR corrected) = 0.008), Muribaculaceae (family; LFC: -0.789, *P* (FDR corrected) = 0.010)/ *Muribaculum* (genus; LFC: -0.897, *P* (FDR corrected) = 0.050), *Lachnospiraceae_bacterium _COE1* (species; LFC: -1.761, *P* (FDR corrected) = 0.019) were significantly decreased in CSC-exposed males born to MvacATCC-treated dams. Additional Spearman correlation analyses revealed that bacterial taxa known to be potent SCFA producers and associated with health benefits correlated positively (marked in green; exact rho and *P*-values are shown in **Figure 6b**) with $CD4^{+}CD25^{+}FoxP3^{+}$ Tregs (in % of all cells), and the Treg suppressive capacity per animal.

After CSC exposure, ANCOM-BC revealed that the relative abundance of Ruminococcaceae (family; LFC: 1.275, *P* (FDR corrected) = 0.008) was significantly increased in CSC males born to MvacATCC-treated mothers (**Figure 6c**). Again, a positive correlation (marked in green; exact rho and *P*-values are shown in **Figure 6d**) was observed between this potent SCFA-producing bacterial taxon and immunological parameters, including $CD4^{+}CD25^{+}FoxP3^{+}$ Tregs (in % of all cells), and the Treg suppressive capacity per animal.

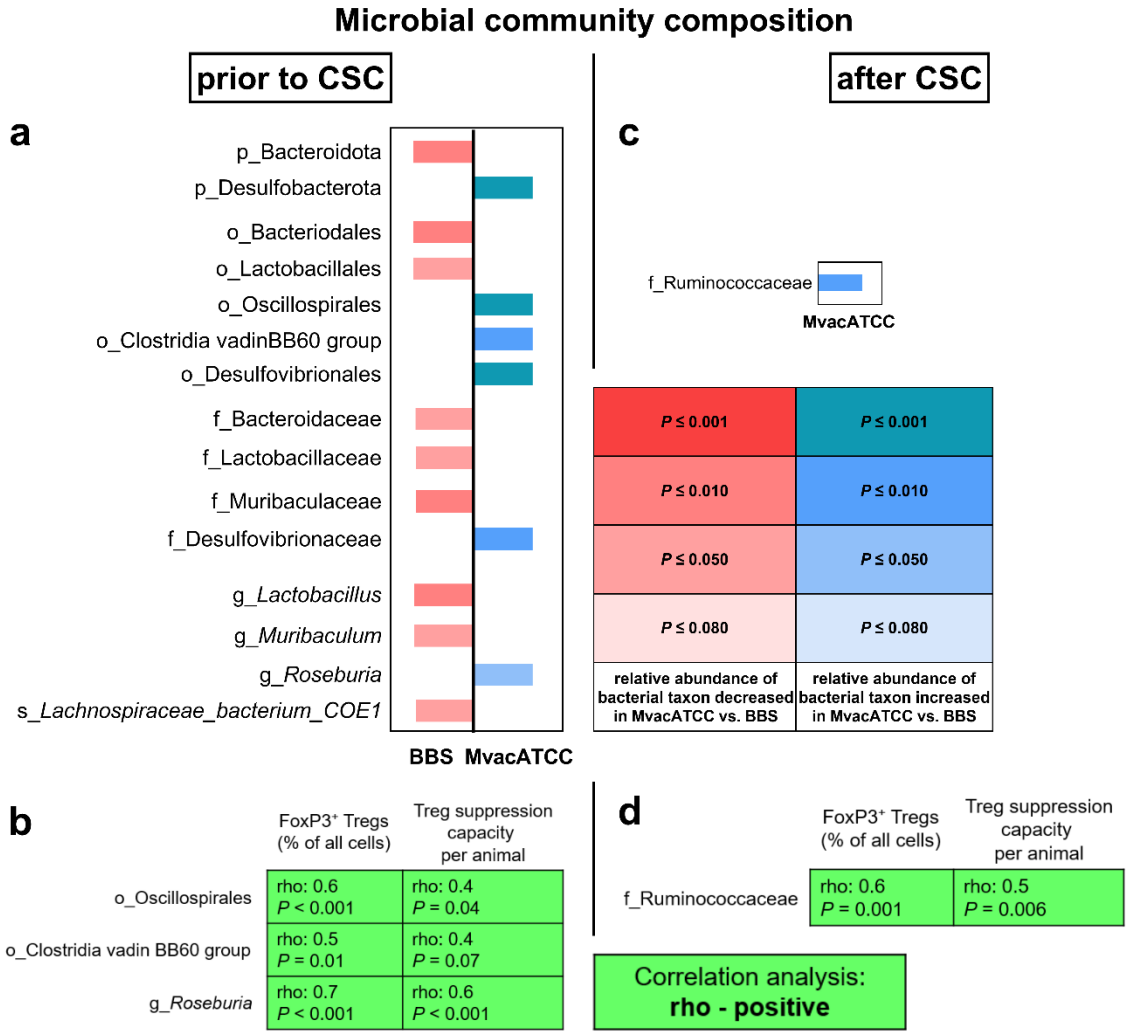


Figure 6 | Intergenerational effects of repeated i.g. administration of *M. vaccae* ATCC 15483T to nulliparous female C57BL/6N mice prior to mating on the community composition of the fecal microbiome prior and after chronic psychosocial stress exposure during adulthood in male offspring (Experiment 1). **a, c)** Relative abundance of bacterial taxa (that were above the established cut-off for microbial abundance analyses (0.1%) in the majority of samples) found to be significantly different between borate-buffered saline (BBS) and M. vaccae ATCC 15483T (MvacATCC)-treated groups (reddish bars: decreased in MvacATCC vs. BBS; blueish bars: increased in MvacATCC vs. BBS) prior **(a)** or after **(b)** chronic psychosocial stress exposure. Taxa sorted by phylum, order, family, genus, and species. **b, d)** Correlation analysis of FoxP3+ Tregs and Treg suppression capacity per animal with orders (o_), and genera (g_) known to be short-chain fatty acid (SCFA) producers prior **(b)** or after **(d)** chronic psychosocial stress exposure.
Adapted from Schiele et al. (2026), licensed under CC BY 4.0.

As the maternal microbiota influences offspring microbial colonization and immune development (Jašarević and Bale, 2019; Kimmel et al., 2024), the fecal microbiome of nulliparous females was analyzed at multiple time points, before mating, on the day of birth, and at weaning, to assess alterations induced by repeated administrations *M. vaccae* ATCC 15483^{T} prior to mating (**Figure 7**) (Schiele et al., 2026).

PERMANOVA analysis of the Bray-Curtis distance matrix revealed significantly different fecal β-diversity between the BBS and *M. vaccae* ATCC 15483^T groups at mating (**Figure 7a**, PERMANOVA; Bray-Curtis; Pseudo-*F* = 2.4053; *P* = 0.016) and at weaning (**Figure 7a**, PERMANOVA; Bray-Curtis; Pseudo-*F* = 1.8161; *P* = 0.010).

Analysis of α-diversity metrics showed significant differences at mating for observed ASVs (**Figure 7b**, MWU: *P* = 0.038) and Chao1 index (**Figure 7d**, MWU: *P* = 0.036), with reduced values in the *M. vaccae* ATCC 15483^T group compared with the respective BBS group. However, a main effect of time was observed for Pielou's evenness (**Figure 7e**, LMM: $F(Time)_{1507,\ 22.61}$ = 3.903, *P* = 0.045), with a significant increase in the *M. vaccae* ATCC 15483^T group from birth to weaning (**Figure 7e**, *post hoc*: *P* = 0.018).

At weaning, ANCOM-BC analysis revealed that the relative abundance of Butyricoccaceae (family; LFC: -1.766, *P* (FDR corrected) = 0.038) and *Desulfovibrio* (genus; LFC: -2.353, *P* (FDR corrected) = 0.043) were significantly decreased in dams treated with *M. vaccae* ATCC 15483^T compared with BBS-treated dams (**Figure 7f**).

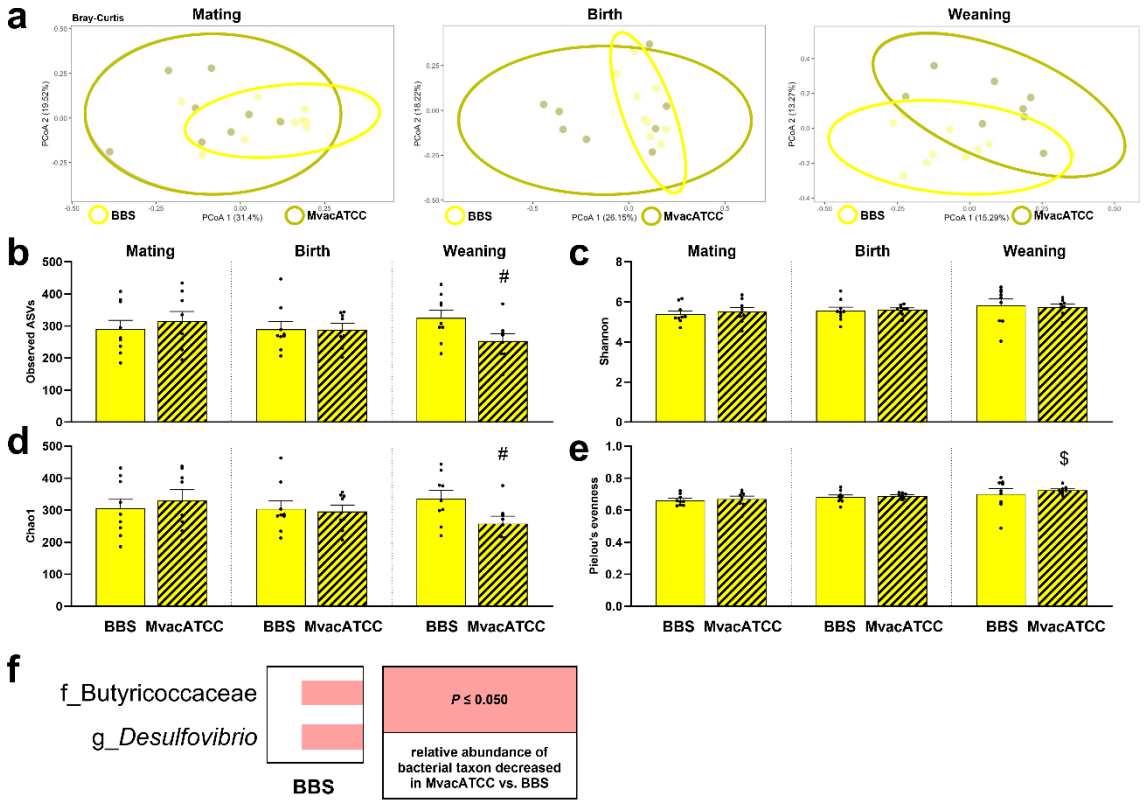


Figure 7 | Effects of repeated i.g. administration of M. vaccae ATCC 15483T to adult, nulliparous female C57BL/6N mice prior to mating on the diversity of their fecal microbiome at mating, birth and weaning (Experiment 1).
a) β-diversity (Bray-Curtis) principal coordinates analysis (PCoA). **b-e)** α-diversity metrics, with **(b)** observed amplicon sequence variants (ASVs), **(c)** Shannon diversity index, **(d)** Chao1 richness estimate (Chao1) and **(e)** Pielou's evenness. Data are presented as mean + SEM including individual values (dots). Non-hatched bars: borate-buffered saline (BBS), hatched bars: *M. vaccae* ATCC 15483^T (MvacATCC).

P ≤ 0.05 vs. respective BBS condition, $ P ≤ 0.05 vs. respective time point birth. *N* = 10 animals per group. **f)** Relative abundance of bacterial taxa (that were above the established cut-off for microbial abundance analyses (0.1 %) in the majority of samples) found to be significantly different between the BBS and *M. vaccae* ATCC 15483^T treated group. Taxa are sorted by family (f_) or genus (g_).
Adapted from Schiele et al. (2026), licensed under CC BY 4.0.

Effects of *M. vaccae* ATCC 15483^T on litter characteristics and maternal behavior

To determine whether the intergenerational effects of *M. vaccae* ATCC 15483^T on the fecal microbiome and stress resilience of male offspring are associated with changes in maternal care or maternal reproductive performance, litter characteristics and maternal behavior were assessed in *M. vaccae* ATCC 15483^T- and BBS-treated dams (**Figure 8**) (Schiele et al., 2026).

At birth, no significant differences were observed between groups in the number of delivered pups (**Figure 8a**) and the body weight per litter (**Figure 8b**). Furthermore, maternal behavior was also comparable between the two groups. Specifically, the percentage of observation intervals spent on nest (in %, with total observation intervals set to 100 %) decreased during the first week after birth (**Figure 8c**), as indicated by a significant main effect of time in both experimental groups (Friedman: BBS: χ^2 (4, 10) = 24.91, $P < 0.001$; MvacATCC: χ^2 (4, 8) = 12.03, $P = 0.017$). *Post hoc* analyses revealed significantly fewer observation intervals spent on nest in the BBS group on lactation days LD4 (Dunn's: $P = 0.007$), LD5 (Dunn's: $P = 0.002$), and LD6 (Dunn's: $P = 0.001$) compared with the first lactation day (LD1). However, no differences were detected between the two maternal treatment groups on any of the observation days (**Figure 8c**). Performance in the pup retrieval test conducted on LD3, including the assessment of the latency to retrieve the first pup (**Figure 8d**) and the latency until all pups were back in nest (**Figure 8e**), also did not differ between the MvacATCC- and BBS-treated dams.

At weaning, no significant differences were detected between groups in litter body weight (**Figure 8f**) or in the number of male and female pups per litter (**Figure 8g**).

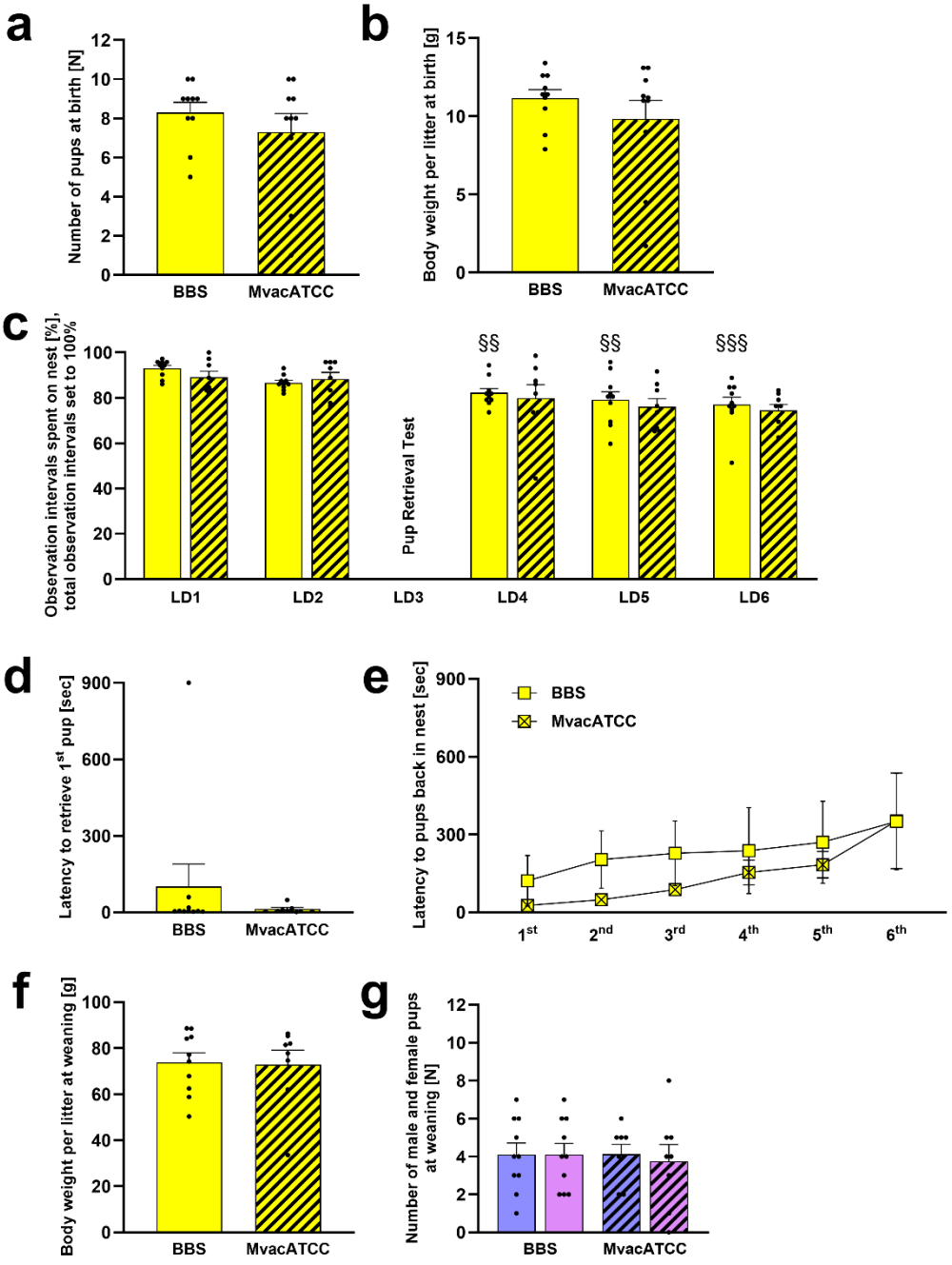


Figure 8 | Effects of repeated i.g. administrations of *M. vaccae* ATCC 15483T to nulliparous C57BL/6N female mice prior to mating on bodyweight of the offspring (Experiment 1) and maternal behavior/ care (Experiment 1).

a) Number of pups per litter at birth. **b)** Body weight per litter at birth. **c)** Percentage (%) of observation intervals (daily observation between lactation day (LD) 1 to LD 6; 72 intervals per day; i.e., 4 intervals per 10 min for 3 h) spent on nest, with total observation intervals set to 100 % (N = 8 litters per group). **d)** Latency to retrieve first pup in the pup retrieval test (PRT). **e)** Latency to retrieve pups back into the nest in the PRT. **f)** Body weight per litter at weaning. **g)** Numbers of pups per sex (males: blue bars, females: purple bars). Data are presented as mean + SEM including individual values (dots). Non-hatched bars: borate-buffered saline (BBS), hatched bars: *M. vaccae* ATCC 15483^T (MvacATCC). §§ $P \leq 0.01$, §§§ $P \leq 0.001$ vs. respective LD1. N = 7 – 10 litters per group. In case individuals had to be excluded due to methodological reasons, this is reported in the material and methods sections 4 and 7.

Adapted from (Schiele et al., 2026), licensed under CC BY 4.0.

To investigate whether repeated i.g. administration of *M. vaccae* ATCC 15483^{T} affected the innate immune system, particularly the complement system transferred *via* the breast milk, relative complement C3 expression levels in mammary gland tissue were determined shortly after birth using RT-qPCR. However, no significant differences were revealed comparing BBS-treated dams with MvacATCC-treated dams of Experiment 2 (**Figure 9**) (Schiele et al., 2026).

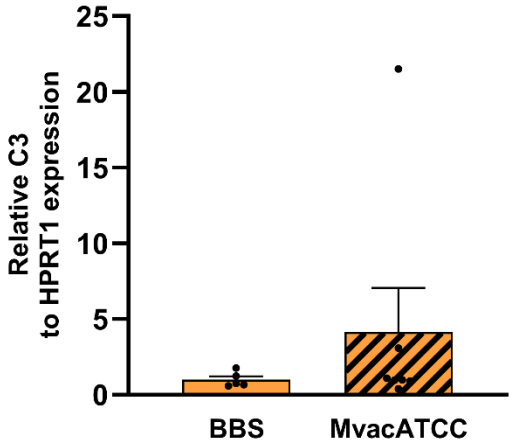


Figure 9 | Effects of repeated i.g. administrations of *M. vaccae* ATCC 15483^{T} to nulliparous female C57BL/6N mice prior to mating on complement C3 expression in mammary gland tissue of dams (Experiment 2). Relative C3 expression in mammary glands. Data are presented as mean + SEM including individual values (dots). Non-hatched bar: borate-buffered saline (BBS), hatched bar: *M. vaccae* ATCC 15483^{T}. $N = 6 – 9$ per group. Adapted from Schiele et al. (2026), licensed under CC BY 4.0.

Effects of *M. vaccae* ATCC 15483^T and prenatal stress on behaviors, physiology, immunology, and bone metabolism in male and female offspring

To determine whether repeated i.g. administration of *M. vaccae* ATCC 15483^T to female mice prior to mating or prenatal stress exposure affected emotional behaviors in male and female offspring, behavioral tests assessing general and/or social anxiety-related behaviors (OF/NO, SPAT, EPM), anhedonia (SPT) and depression-like (FST) behavior were performed (**Figure 10**) (Schiele et al., 2025).

In the OF/NO test, statistical analysis revealed a main effect of stress in male offspring for the parameter distance moved during the OF condition (**Figure 10a**, 2WayANOVA, $F(Stress)_{1,44} = 4.754$; $P = 0.035$). However, entries into inner zone in OF (**Figure 10b**) and entries into contact zone during NO exploration (**Figure 10c**) were comparable between experimental male groups. In females, only MvacATCC-PS mice travelled significantly more compared to the BBS-PS group (**Figure 10d**, 2WayANOVA, $F(Treatment)_{1,44} = 4.529$; $P = 0.039$; Bonferroni: $P = 0.039$), while entries into inner zone in the OF (**Figure 10e**) did not differ between female experimental groups. However, a main effect for the factor was found for entries into the contact zone during the NO condition stress (**Figure 10f**, 2WayANOVA, $F(Stress)_{1,44} = 4.505$; $P = 0.039$).

For the social preference assessed by SPAT, MvacATCC-PS males were significantly affected by maternal *M. vaccae* ATCC15483^T treatment (**Figure 10g**, MWU: $P = 0.001$) and showed a trend for an effect of prenatal stress (**Figure 10g**, MWU: $P = 0.060$) compared to the respective controls, whereas social preference in females was neither affected by maternal *M. vaccae* ATCC15483^T treatment nor by prenatal stress (**Figure 10h**).

Statistical analysis of the EPM (**Figures 10i-l**) revealed significant differences only in the time spent in the open arms (OA; relative to time spent in all arms) in females (**Figure 10l**). In detail, prenatal stress significantly reduced time in OAs in females of both BBS- (**Figure 10l**, MWU: $P = 0.017$) and MvacATCC-treated dams (**Figure 10l**, MWU: $P = 0.006$). This reduction was even more pronounced in offspring of MvacATCC-treated dams (**Figure 10l**, MWU: $P = 0.045$).

The assessment of anhedonic-like behavior revealed that, in males, saccharin preference was significantly reduced by maternal *M. vaccae* ATCC 15483^T in PS-exposed offspring (**Figure 10m**, MWU: $P = 0.013$). In females, saccharine preference was significantly reduced by prenatal stress in offspring of MvacATCC-treated dams (**Figure 10n**, MWU: $P = 0.043$).

The FST was used to assess depression-like behavior. No effects were found in males (**Figure 10o**). In contrast, a main effect for maternal *M. vaccae* ATCC 15483^{T} treatment was revealed in female offspring (**Figure 10p**, 2WayANOVA, F(Treatment)$_{1,44}$ = 5.650; P = 0.022).

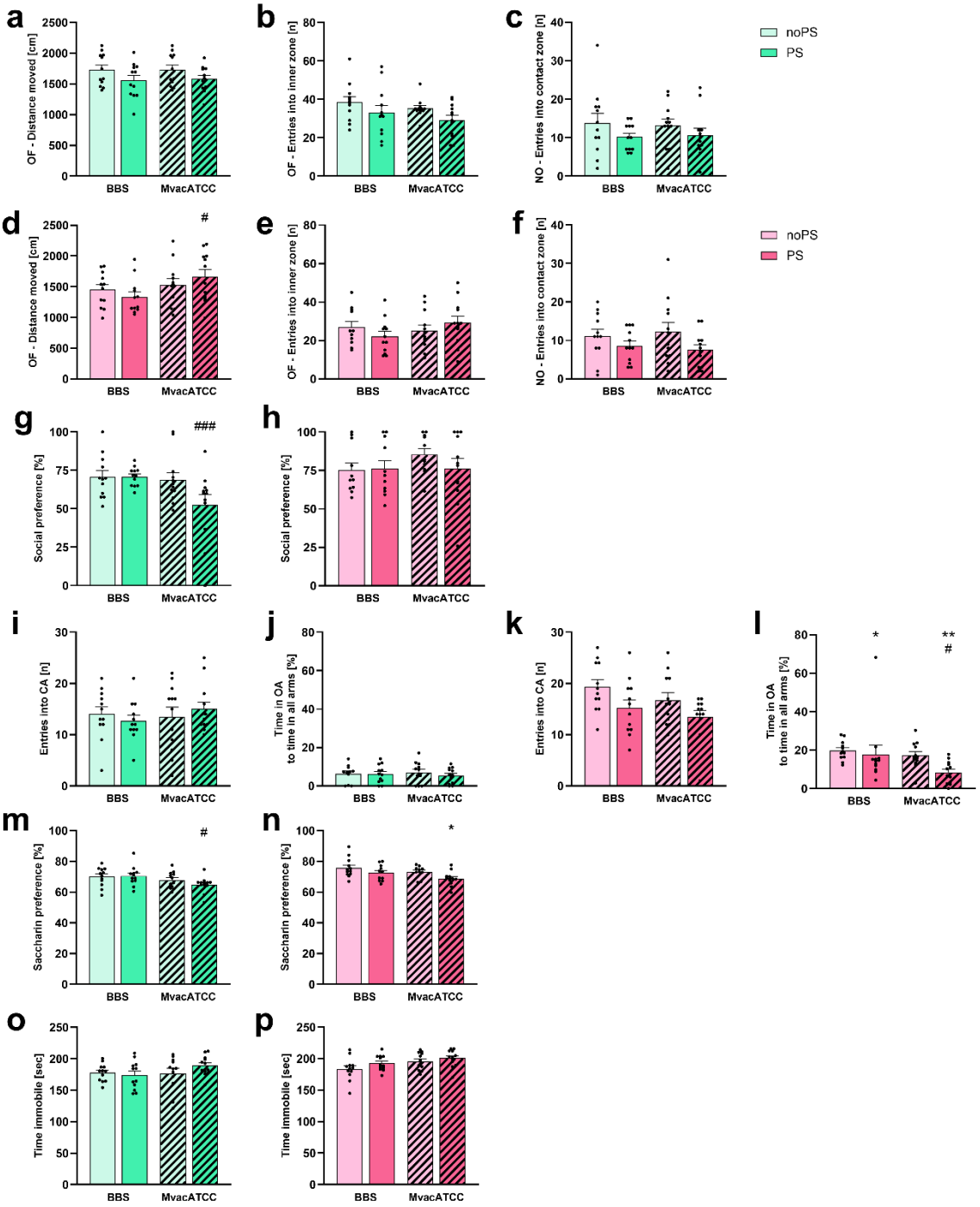


Figure 10 | Effects of *M. vaccae* ATCC 15483^{T} and prenatal stress on behavior in male and female offspring (Experiment 3).

a-p) show data from experimental male **(a-b, g, i, j, m, o)** and female **(d-f, h, k, l, n, p)** offspring exposed to prenatal stress (PS; dark colored bars) or not (noPS; light colored bars). In the open field (OF)/novel object (NO) test, **a, d)** Distance moved and **b, e)** Entries into inner zone in the OF, and

c, f) Entries into contact zone in the NO. In the social preference/avoidance test (SPAT), **g, h)** Social preference (time spent in direct contact zone (social) divided by total time spent in direct contact zone (empty + social)). In the elevated plus maze (EPM), **i, k)** Entries into closed arms (CA), and **j, l)** Time in open arms (OA). In the saccharin preference test (SPT), **m, n)** Saccharin preference (volume drunk (saccharin solution) divided by total volume drunk (tap water + saccharin). In the forced swim test (FST), **o, p)** Time immobile. Data are presented as mean + SEM including individual values (dots). Non-hatched bar: borate-buffered saline (BBS), hatched bar: *M. vaccae* ATCC 15483^T. $N = 12$ per group. * $P \leq 0.05$, ** $P \leq 0.010$ vs. respective noPS condition; # $P \leq 0.05$, ### $P \leq 0.001$ vs. respective BBS condition. In case individuals had to be excluded due to methodological reasons, this is reported in the material and methods section 11. Adapted from Schiele et al. (2025), licensed under CC BY 4.0.

To investigate whether *M. vaccae* ATCC 15483^T can also intergenerationally protect male and female offspring from potential negative physiological and immunological consequences of prenatal stress, well-established stress-sensitive organs, such as the adrenal glands, and thymus, as well as plasma CORT levels, and splenic IgG protein levels, were examined (**Figure 11**) (Schiele et al., 2025).

In male offspring, adrenal weight (**Figure 11a**), thymus weight (**Figure 11b**), and plasma CORT levels under basal conditions (**Figure 11c**), as well as after acute restraint stress (RST), which were significantly increased vs. basal levels (**Figure 11c**, Wilcoxon: BBS-noPS: $P = 0.004$, BBS-PS, MvacATCC-noPS, and MvacATCC-PS: $P = 0.001$), did not differ between experimental groups. In female offspring, relative adrenal weight tended to be increased by maternal *M. vaccae* ATCC 15483^T treatment (**Figure 11d**, 2WayANOVA: $F(\text{Treatment})_{1,43} = 6.655$, $P = 0.013$; Bonferroni: $P = 0.056$). However, relative thymus weight (**Figure 11e**) and plasma CORT levels under basal conditions (**Figure 11f**), as well as after RST, which were significantly increased vs. basal levels (**Figure 11f**, Wilcoxon: in all groups: $P = 0.001$), were comparable between all female groups.

Splenic IgG protein expression levels were analyzed using Western blot. Statistical analysis revealed that PS significantly increased splenic IgG levels in males of BBS-treated dams (**Figure 11g**, 2WayANOVA: $F(\text{Stress})_{1,44} = 11.75$; $P = 0.001$, Bonferroni: $P = 0.027$) compared to respective BBS-noPS males. Furthermore, repeated i.g. administration of *M. vaccae* ATCC 15483^T to female mice before mating tended to increase splenic IgG levels in non-prenatally stressed male offspring (**Figure 11g**, 2WayANOVA: $F(\text{Stress})_{1,44} = 11.75$; $P = 0.001$, Bonferroni: $P = 0.069$) compared to respective BBS-noPS males. Splenic IgG protein levels in female offspring were comparable between all experimental groups (**Figure 11h**).

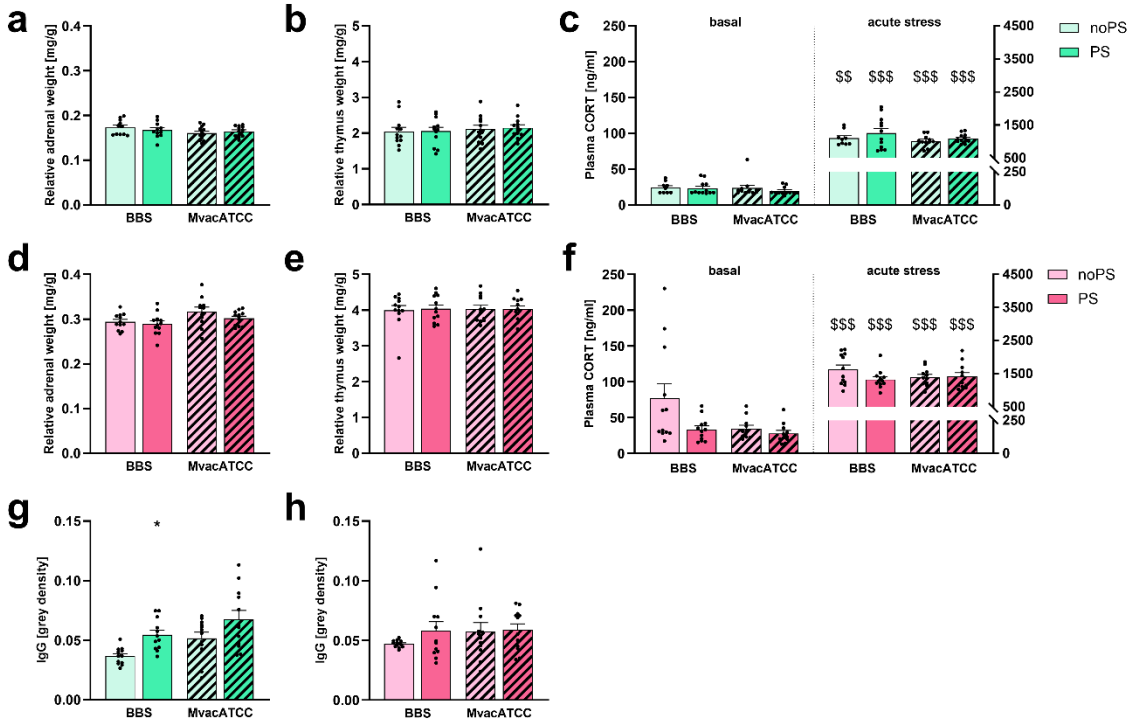


Figure 11 | Effects of *M. vaccae* ATCC 15483^T and prenatal stress on physiology and immunology in male and female offspring (Experiment 3).
a-h) show data from experimental male **(a-c, g)** and female **(d-f, h)** offspring exposed to prenatal stress (PS; dark colored bars) or not (noPS; light colored bars). Relative **a, d)** adrenal and **b, e)** thymus weight. **c, f)** Plasma corticosterone (CORT) levels. Splenic **g, h)** immunoglobulin (Ig)G levels. Data are presented as mean + SEM including individual values (dots). Non-hatched bar: borate-buffered saline (BBS), hatched bar: *M. vaccae* ATCC 15483^T. $N = 12$ per group. * $P \leq 0.05$ vs. respective noPS condition; §§ $P \leq 0.01$, §§§ $P \leq 0.001$ vs. respective basal condition. In case individuals had to be excluded due to methodological reasons, this is reported in the material and methods section 17. Adapted from Schiele et al. (2025), licensed under CC BY 4.0.

As chronic psychosocial stress is known to affect long-bone growth in adult male mice (Foertsch et al., 2017b), it was assessed whether PS also affects bone parameters and whether repeated i.g. administration of *M. vaccae* ATCC 15483^T to females prior to mating intergenerationally protects male and female offspring from potential negative consequences of PS (**Figure 12**) (Schiele et al., 2025).

The assessment of femur length in male offspring revealed no significant differences between experimental groups (**Figure 12a**). However, the trabecular bone volume/ tissue volume ratio (BV/TV) was significantly reduced in BBS-PS males compared to the respective noPS group (**Figure 12b**, 2WayANOVA: $F(Stress)_{1,44} = 10.68$; $P = 0.002$, Bonferroni: $P = 0.013$). In contrast, femur length was significantly increased in PS-exposed females of MvacATCC-treated mothers compared to respective noPS-exposed mice (**Figure 12c**, 2WayANOVA: $F(Interaction)_{1,43} = 4.138$; $P = 0.048$, Bonferroni: $P = 0.014$), while BV/TV in female offspring was comparable between all groups (**Figure 12d**).

Furthermore, several bone parameters were reduced in PS-exposed male offspring of BBS-treated females, including trabecular tissue mineral density (TbTMD, **Figure 12e**, 2WayANOVA, $F(Stress)_{1,44} = 8.097$; $P = 0.007$, Bonferroni: $P = 0.005$), trabecular thickness (TbTh, **Figure 12f**, 2WayANOVA, 1 outlier in the BBS-noPS group: $F(Stress)_{1,43} = 15.08$; $P \leq 0.001$, Bonferroni: $P = 0.007$), and trabecular number (TbN, **Figure 12g**, MWU: $P = 0.029$). Additionally, PS exposure significantly decreased TbTh in male offspring of MvacATCC-treated dams (**Figure 12f**, 2WayANOVA, 1 outlier in the BBS-noPS group: $F(Stress)_{1,43} = 15.08$; $P \leq 0.001$, Bonferroni: $P = 0.042$), showed a trend toward increased trabecular separation (TbSp, **Figure 12h**, 2WayANOVA: $F(Stress)_{1,44} = 4.758$; $P = 0.035$, Bonferroni: $P = 0.052$), and significantly increased cortical thickness (cTh, **Figure 12n**, MWU: $P = 0.021$) in PS-exposed males of MvacATCC-treated mothers.

Maternal *M. vaccae* ATCC 15483^T treatment significantly increased TbTMD intergenerationally in PS-exposed male offspring (**Figure 12e**, 2WayANOVA, $F(Treatment)_{1,44} = 7.771$; $P = 0.008$, Bonferroni: $P = 0.006$). It also significantly increased TbTh in both the PS-exposed and control groups (**Figure 12f**, 2WayANOVA, $F(Treatment)_{1,43} = 15.48$; $P < 0.001$, Bonferroni: noPS: $P = 0.043$, PS: $P = 0.005$). The only bone parameter that was not affected by either PS or maternal M. vaccae ATCC 15483^T, was cortical tissue mineral density (cTMD, **Figure 12m**).

In female offspring, with the exception of femur length, the investigated bone parameters, including TbTMD (**Figure 12i**), TbTh (**Figure 12j**), TbN (**Figure 12k**), TbSp (**Figure 12l**), cTMD (**Figure 12o**), and cTh (**Figure 12p**), were comparable between all experimental groups.

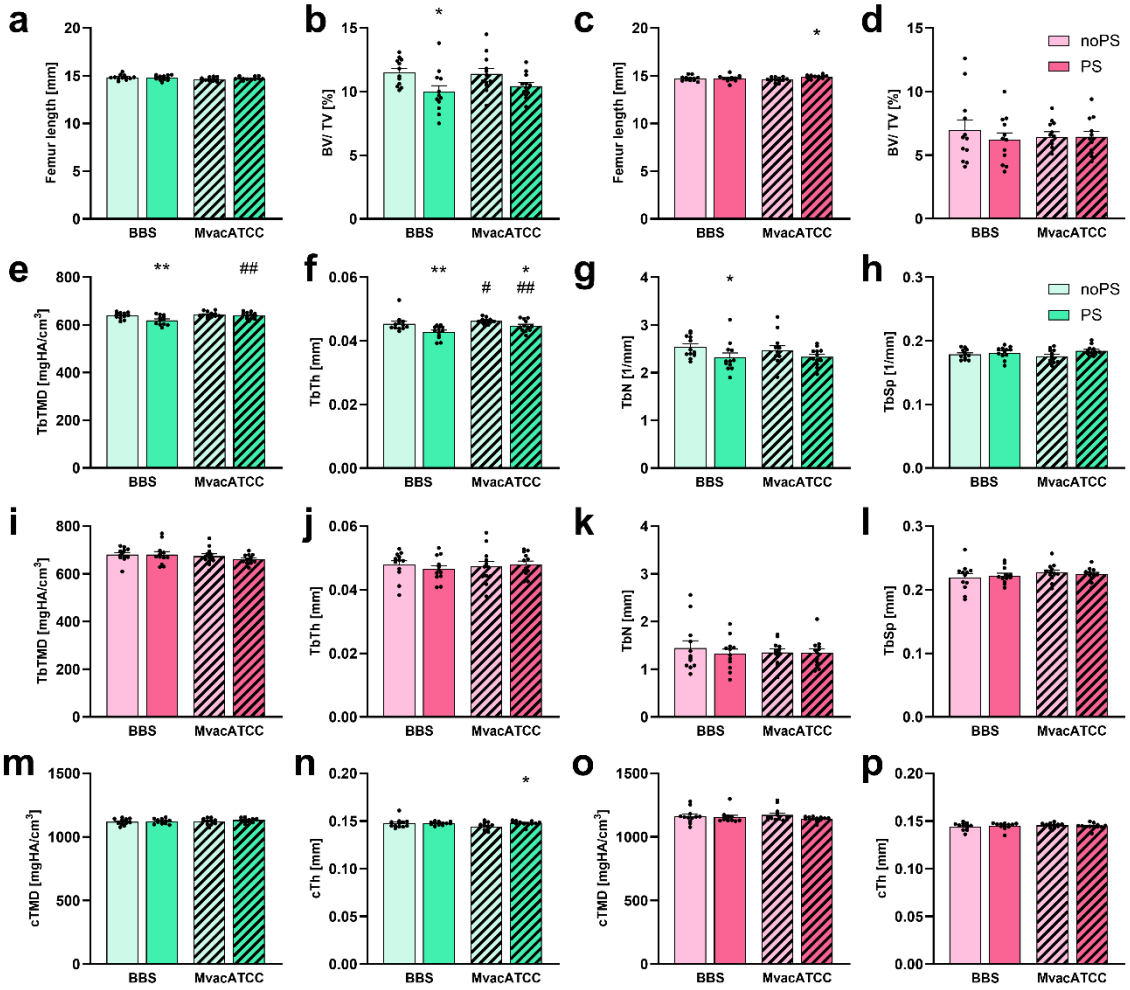


Figure 12 | Effects of *M. vaccae* ATCC 15483^T and prenatal stress on bone metabolism in male and female offspring (Experiment 3). a-p) show data from experimental male **(a, b, e-h, m, n)** and female **(c, d, i-l, o, p)** offspring exposed to prenatal stress (PS; dark colored bars) or not (noPS; light colored bars). Relative **a, c)** Femur length. **b, d)** Bone volume/tissue volume ratio (BV/TV). **e, i)** Trabecular tissue mineral density (TbTMD). **f, j)** Trabecular thickness (TbTh). **g, k)** Trabecular number (TbN). **h, l)** Trabecular separation (TbSp). **m, o)** Cortical tissue mineral density (cTMD). **n, p)** Cortical thickness (cTh). Data are presented as mean + SEM including individual values (dots). Non-hatched bar: borate-buffered saline (BBS), hatched bar: *M. vaccae* ATCC 15483^T. $N = 12$ per group. * $P \leq 0.05$, ** $P \leq 0.01$ vs. respective noPS condition; # $P \leq 0.05$, ## $P \leq 0.01$ vs. respective BBS condition. Adapted from Schiele et al. (2025), licensed under CC BY 4.0.

Effects of *M. vaccae* ATCC 15483^T and prenatal stress on behaviors, physiology, immunology, and bone metabolism in single-housed adult male offspring

To assess whether the effects of maternal *M. vaccae* ATCC 15483^T treatment and PS exposure are long-lasting, general and social anxiety-related behaviors were investigated in single-housed adult male offspring of Experiment 4 (**Figure 13**) (Schiele et al., 2025).

In the SPAT, statistical analysis revealed a main effect of treatment (**Figure 13a**, 2WayANOVA, 1 outlier in the BBS-PS group, $F(\text{Treatment})_{1,38} = 5.412$, $P = 0.025$). In contrast, PS, independent of maternal *M. vaccae* ATCC 15483^T treatment, significantly reduced the total distance moved during the OF exploration (**Figure 13b**, MWU: BBS: $P = 0.039$, MvacATCC: $P = 0.025$) in these adult males.

Entries into the inner zone (OF; **Figure 13c**) and into the contact zone (NO; **Figure 13d**) did not differ between experimental groups.

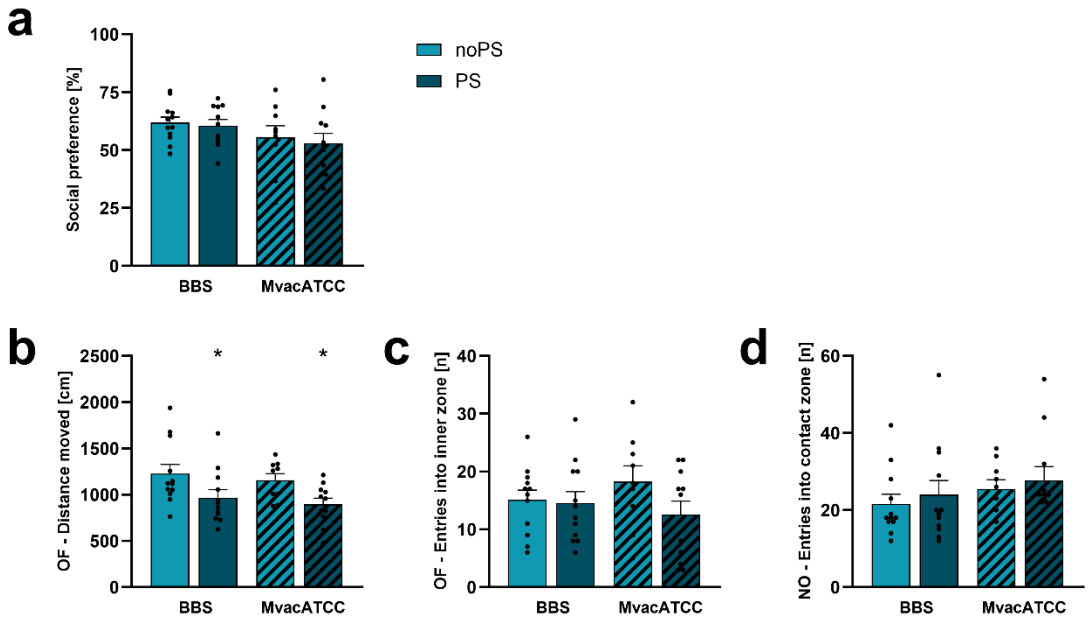


Figure 13 | Effects of *M. vaccae* ATCC 15483^T and prenatal stress on behavior in adult male offspring (Experiment 4).
In the social preference/avoidance test (SPAT), **a)** Social preference (time spent in direct contact zone (social) divided by total time spent in direct contact zone (empty + social)). In the open field (OF)/novel object (NO) test, **b)** Distance moved and **c)** Entries into inner zone in the OF, and **d)** Entries into contact zone in the NO. Data are presented as mean + SEM including individual values (dots). Light colored bar: no prenatal stress exposure (noPS), dark colored bar: prenatal stress exposure (PS). Non-hatched bar: borate-buffered saline (BBS), hatched bar: *M. vaccae* ATCC 15483^T. $N = 9 – 12$ per group. * $P \leq 0.05$ vs. respective noPS condition. Adapted from Schiele et al. (2025), licensed under CC BY 4.0.

Furthermore, potential long-lasting effects of maternal *M. vaccae* ATCC 15483^T treatment and PS exposure on physiological and immunological parameters were assessed in more mature adult male offspring of Experiment 4 (**Figure 14**) (Schiele et al., 2025).

Physiological parameters, including relative adrenal weight (**Figure 14a**) and the relative thymus weight (**Figure 14b**), but also plasma CORT concentrations (**Figure 14c**), were not affected by either repeated i.g. administration of *M. vaccae* ATCC 15483^T or PS exposure. In contrast, immunological parameters showed significant differences in adult male offspring.

For instance, splenic IgG protein levels were significantly increased in PS-exposed adult males of BBS-treated dams compared with their respective noPS controls (**Figure 14d**, 2WayANOVA: $F(Stress)_{1,40} = 11.26$; $P = 0.002$, $F(PS*Treatment)_{1,40} = 6.747$; $P = 0.013$, Bonferroni: $P = 0.014$). Moreover, maternal *M. vaccae* ATCC 15483^T treatment tended to increase splenic IgG protein levels in adult males not exposed to PS when compared with the respective BBS-noPS group (**Figure 14d**, 2WayANOVA: $F(Stress)_{1,40} = 11.26$; $P = 0.002$, $F(PS*Treatment)_{1,40} = 6.747$; $P = 0.013$, Bonferroni: $P = 0.066$).

In addition, delta (LPS-basal) cell viability of isolated splenocytes in the absence of CORT (0 µM) was significantly increased in PS-exposed males born to BBS-treated dams (**Figure 14e**, MWU: $P = 0.014$) vs. their respective noPS controls. In contrast, PS significantly reduced splenic *ex vivo* delta cell viability in adult males born to MvacATCC-treated mothers (**Figure 14e**, MWU: $P = 0.038$) compared to the noPS-exposed males of the same maternal treatment group. Conversely, maternal *M. vaccae* ATCC 15483^T treatment intergenerationally increased *ex vivo* delta cell viability of splenocytes in adult males not exposed to PS (**Figure 14e**, MWU: $P = 0.003$) compared with the respective BBS control group.

Furthermore, chemotactic activity of isolated neutrophils was significantly increased in adult male mice exposed to PS and born to BBS-treated mothers (**Figure 14f**, MWU: $P = 0.002$) when compared to respective non-prenatally stressed males. This PS-induced was intergenerationally prevented by maternal *M. vaccae* ATCC treatment (**Figure 14f**, MWU: $P = 0.015$).

Lastly, plasma lipopolysaccharide binding protein (LBP) levels in males born to BBS-treated dams were significantly decreased by PS exposure (**Figure 14g**, MWU: $P = 0.033$) compared with the respective noPS group.

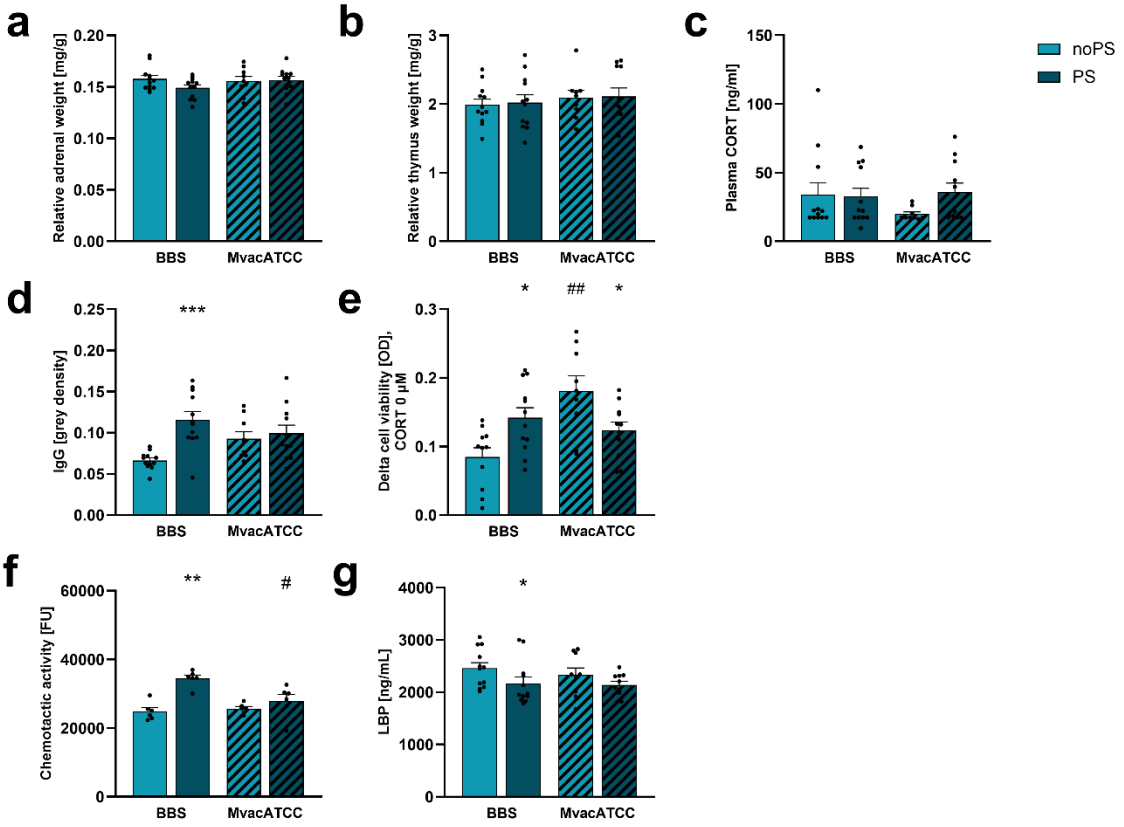


Figure 14 | Effects of *M. vaccae* ATCC 15483^T and prenatal stress on physiology and immunology in adult male offspring (Experiment 4).
Relative **a)** adrenal, and **b)** thymus weight. **c)** Plasma corticosterone (CORT) levels. **d)** Splenic immunoglobulin (Ig)G protein levels. **e)** Delta (LPS-basal) cell viability (CORT 0 µM). **f)** Neutrophil chemotactic activity. **g)** Plasma lipopolysaccharide binding protein (LBP) concentrations. Data are presented as mean + SEM including individual values (dots). Light colored bar: no prenatal stress exposure (noPS), dark colored bar: prenatal stress exposure (PS). Non-hatched bar: borate-buffered saline (BBS), hatched bar: *M. vaccae* ATCC 15483^T. $N = 9 – 12$ per group. In case individual samples had to be excluded due to methodological reasons, this is reported in the respective materials and methods section 26. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ vs. respective noPS condition. # $P \leq 0.05$, ## $P \leq 0.01$ vs. respective noPS condition.
Adapted from Schiele et al. (2025), licensed under CC BY 4.0.

To investigate whether the effects of maternal *M. vaccae* ATCC 15483^T treatment and PS on bone metabolism persist into adulthood, the bone parameters assessed in male and female offspring of Experiment 3 were also analyzed in adult male offspring of Experiment 4 (**Figure 15**), as only males were affected by PS and M. vaccae ATCC 15483^T (Schiele et al., 2025). However, no significant differences between adult male groups were observed for any of the parameters analyzed, including femur length (**Figure 15a**), BV/TV (**Figure 15b**), TbTMD (**Figure 15c**), TbTh (**Figure 15d**), TbN (**Figure 15e**), TbSp (**Figure 15f**), cTMD (**Figure 15g**), cTh (**Figure 15h**).

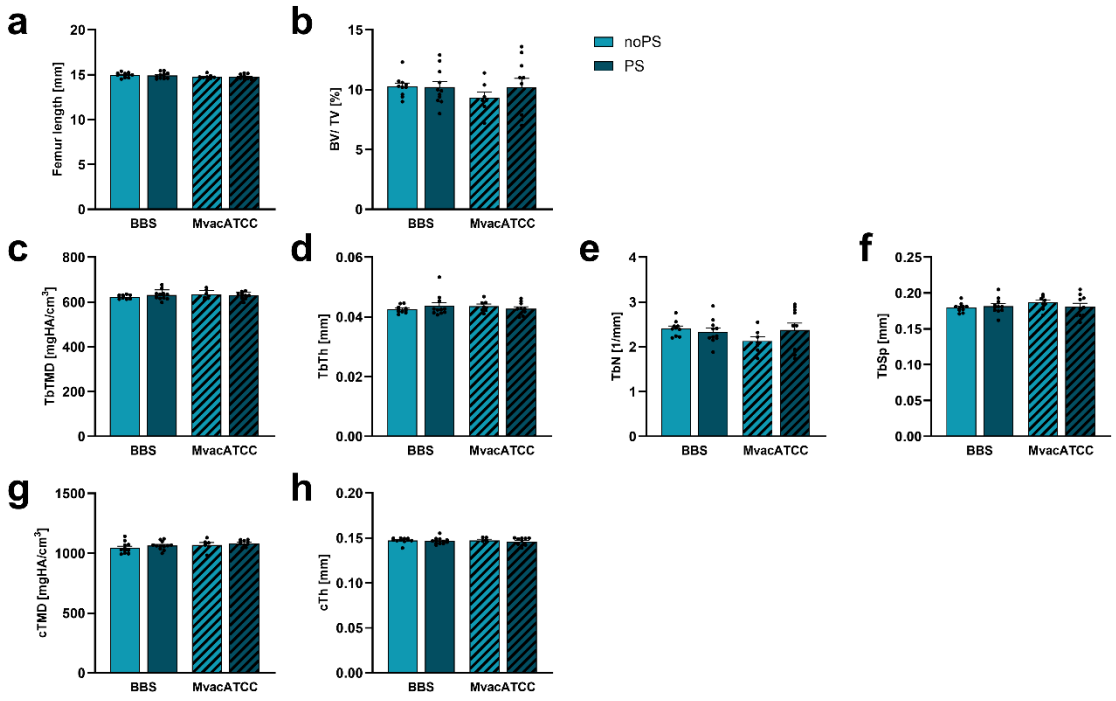


Figure 15 | Effects of *M. vaccae* ATCC 15483^T and prenatal stress on bone metabolism in adult male offspring (Experiment 4).
a) Femur length **b)** Bone volume/tissue volume ratio (BV/TV). **c)** Trabecular tissue mineral density (TbTMD). **d)** Trabecular thickness (TbTh). **e)** Trabecular number (TbN). **f)** Trabecular separation (TbSp). **g)** Cortical tissue mineral density (cTMD). **h)** Cortical thickness (cTh). Data are presented as mean + SEM including individual values (dots). Light colored bar: no prenatal stress exposure (noPS), dark colored bar: prenatal stress exposure (PS). Non-hatched bar: borate-buffered saline (BBS), hatched bar: *M. vaccae* ATCC 15483^T. $N = 9 – 12$ per group. In case individual samples had to be excluded due to methodological reasons, this is reported in the respective materials and methods sections 20 and 21.
Adapted from Schiele et al. (2025), licensed under CC BY 4.0.

Effects of *M. vaccae* ATCC 15483^T and prenatal stress on immunology in male pups

To determine when the effects of repeated i.g. administration of *M. vaccae* ATCC 15483^T and prenatal stress exposure on splenic IgG protein levels first appear in male offspring, whole spleens from male pups were analyzed for IgG protein levels between LD 1 and LD 3 (Experiment 5). Western blot analysis did not reveal significant differences in splenic IgG protein levels between the experimental male pup groups (**Figure 16**) (Schiele et al., 2025).

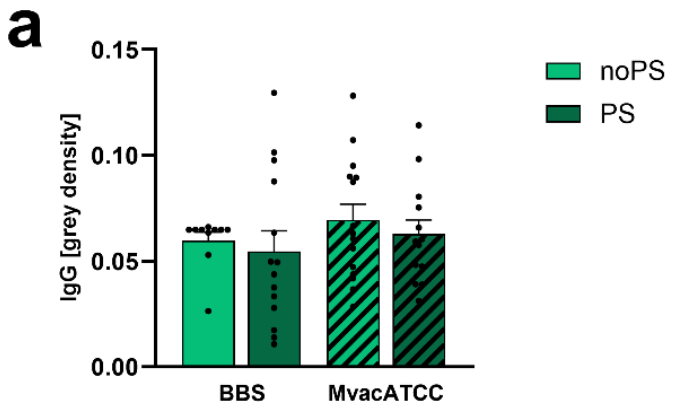


Figure 16 | Effects of *M. vaccae* ATCC 15483^T and prenatal stress on splenic IgG expression in male pups (Experiment 5). Data are presented as mean + SEM including individual values (dots). Light colored bar: no prenatal stress exposure (noPS), dark colored bar: prenatal stress exposure (PS). Non-hatched bar: borate-buffered saline (BBS), hatched bar: *M. vaccae* ATCC 15483^T. $N = 10 – 15$ per group.
Adapted from Schiele et al. (2025), licensed under CC BY 4.0.

Effects of *M. vaccae* ATCC 15483^T and prenatal stress on litter characteristics and maternal behavior

To determine whether the intergenerational effects of *M. vaccae* ATCC 15483^T and prenatal stress on immunological and bone-related parameters in male offspring are associated with changes in maternal care or maternal reproductive performance, litter characteristics and maternal behavior were assessed dams repeatedly administered with *M. vaccae ATCC* 15483^T and BBS (**Figure 17**) (Schiele et al., 2025).

At birth, no significant differences were observed between groups in the number of delivered pups (**Figure 17a**) or the body weight per litter (**Figure 17b**). In contrast, significant differences between the experimental maternal groups were detected for some maternal behavior parameters.

In detail, the percentage of observation intervals spent on nest (in %, with total observation intervals set to 100 %) decreased during the first week after birth (**Figure 17c**), as indicated by a significant main effect of time in all groups (Friedman: BBS-noPS: χ^2 (4, 10) = 24.91, P < 0.001; BBS-PS: χ^2 (4, 9) = 14.32, P = 0.006; MvacATCC-noPS: χ^2 (4, 8) = 12.03, P = 0.017; MvacATCC-PS: χ^2 (4, 9) = 21.88, P < 0.001). *Post hoc* analyses revealed significantly fewer observation intervals spent on nest in the BBS-noPS group on LD4 (Dunn's: P = 0.007), LD5 (Dunn's: P = 0.002), and LD6 (Dunn's: P = 0.001), in the BBS-PS group on LD6 (Dunn's: P = 0.022), and in the MvacATCC-PS group on LD4 (Dunn's: P = 0.029), LD5 (Dunn's: P = 0.010), and LD6 (Dunn's: P = 0.001), all compared with the first lactation day (LD1). A trend was additionally found in the MvacATCC-PS group for LD6 compared with LD2 (Dunn's: P = 0.058). However, group differences in observation intervals spent on nest were detected only on LD2. This parameter was significantly higher in the BBS-PS female group compared with the BBS-noPS group (**Figure 17c**, MWU: P = 0.039), and tended to be lower in the MvacATCC-PS group compared with respective BBS-PS females (**Figure 17c**, MWU: P = 0.072).

In the PRT conducted on LD3, dams repeatedly treated with *M. vaccae* ATCC 15483^T and exposed to pregnancy stress showed a significantly increased latency to retrieve first pup (**Figure 17d**, MWU: P = 0.027) compared with dams exposed to pregnancy stress but treated with BBS prior to mating. The parameter "latency to pups back in nest" was unaffected (**Figure 17e**).

At weaning, no significant differences between groups were detected in litter body weight (**Figure 17f**) or in the number of male and female pups per litter (**Figure 17g**).

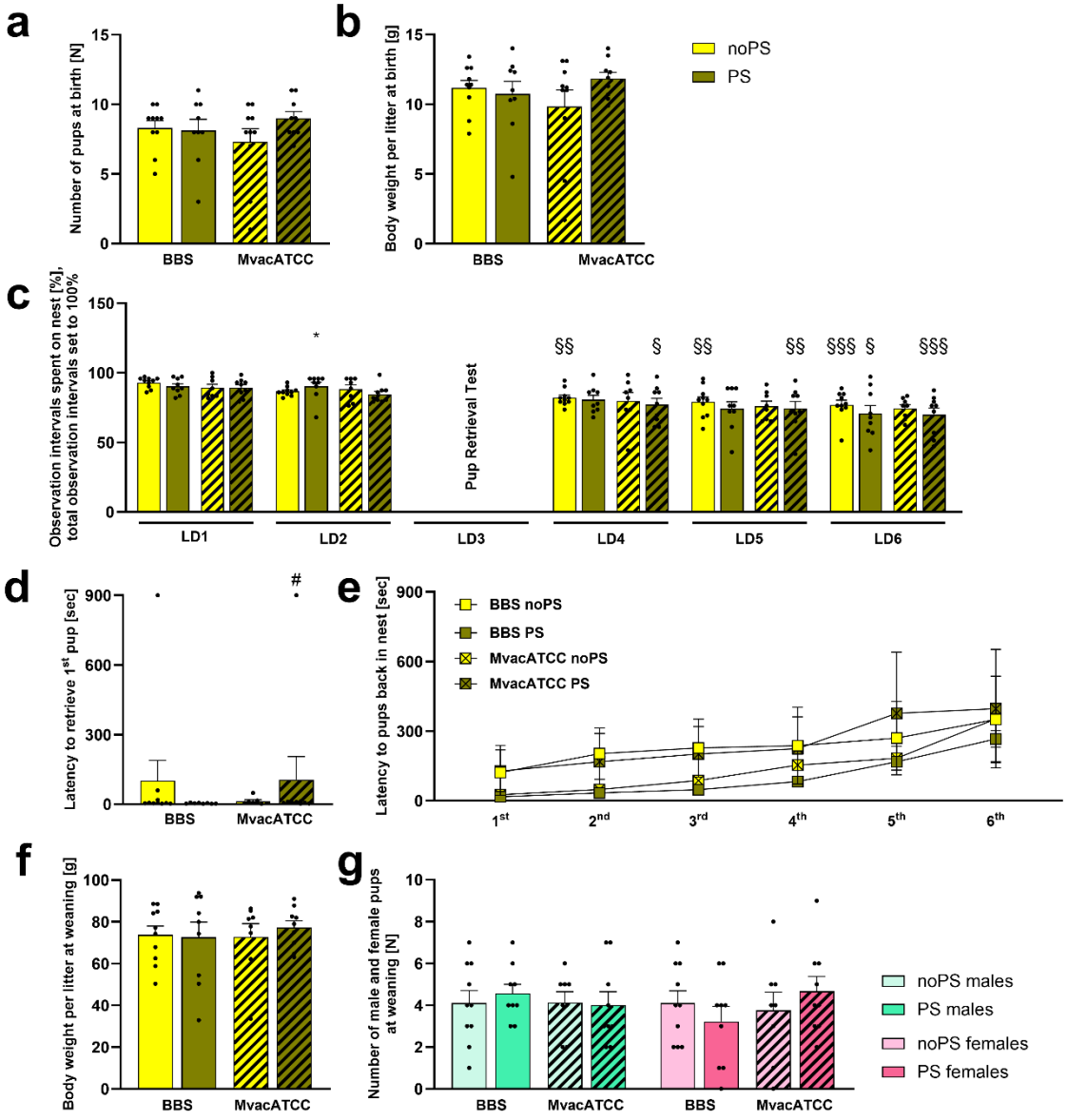


Figure 17 | Effects of repeated i.g. administrations of *M. vaccae* ATCC 15483T to nulliparous C57BL/6N female mice prior to mating and prenatal stress on bodyweight of the offspring (Experiment 4) and maternal behavior/care (Experiment 4).
a) Number of pups per litter at birth. **b)** Body weight per litter at birth. **c)** Percentage (%) of observation intervals (daily observation between lactation day (LD) 1 to LD 6; 72 intervals per day; i.e., 4 intervals per 10 min for 3 h) spent on nest, with total observation intervals set to 100 % (N = 8 litters per group). **d)** Latency to retrieve first pup in the pup retrieval test (PRT). **e)** Latency to retrieve pups back into the nest in the PRT. **f)** Body weight per litter at weaning. **g)** Numbers of pups per sex (males: mint bars, females: rose bars). Data are presented as mean + SEM including individual values (dots). Light colored bars: no prenatal stress (noPS) exposure; dark colored bars: prenatal stress (PS) exposure. Non-hatched bars: borate-buffered saline (BBS), hatched bars: *M. vaccae* ATCC 15483^T (MvacATCC). § P ≤ 0.05, §§ P ≤ 0.01, §§§ P ≤ 0.001 vs. respective LD1. N = 8 – 10 litters per group. In case individuals had to be excluded due to methodological reasons, this is reported in the material and methods sections 4 and 7.
Adapted from Schiele et al. (2025), licensed under CC BY 4.0.

Discussion

The prevalence of chronic stress-associated somatic and mental disorders, including depression and PTSD, has increased in recent decades, particularly in urban environments of highly industrialized countries (Piao et al., 2024; Xu et al., 2023a; Xu et al., 2023b). These disorders are associated with chronic low-grade inflammation and a dysregulated immune system (Gola et al., 2013; Iwata et al., 2013; Kivimaki et al., 2014; Lindqvist et al., 2014; Liu et al., 2022b; Miller and Raison, 2016; Reber, 2012; Taylor et al., 2016). The "Old Friends" hypothesis proposes that reduced exposure to immunoregulatory microorganisms contributes to this increase by impairing proper immune function (McDade et al., 2013; Miller and Raison, 2016; Rook, 2013; Rook, 2023). In line with this hypothesis, previous preclinical studies using non-pathogenic rapid-growing mycobacteria, such as *M. vaccae* NCTC 11659 and *M. vaccae* ATCC 15483^T, have demonstrated immunoregulatory and stress-protective effects when administered *via* different routes (e.g., s.c. or i.g.), either before or during stressor exposure (Amoroso et al., 2020a; Amoroso et al., 2019; Desmond et al., 2024; Frank et al., 2018a; Gebauer et al., 2025; Langgartner et al., 2023a; Loupy et al., 2021; Mazzari et al., 2023; Reber et al., 2016b; Schimmele et al., 2025). Epidemiological evidence further suggests that the timing and duration of microbial exposure are critical determinants of immunological outcome (Anumula et al., 2024; Koren et al., 2024). In particular, early life microbial exposure during pre- and postnatal development appears to play a crucial role in shaping immune function and stress resilience in later life (Ege et al., 2008; Schaub et al., 2009). However, it remains unclear to date whether maternal exposure to immunoregulatory microorganisms prior to conception can confer intergenerational stress resilience. Therefore, the present study investigated whether maternal exposure to a heat-killed preparation of *M. vaccae* ATCC 15483^T prior to mating enhance stress resilience in the male and female offspring.

Nulliparous female C57BL/6N mice were repeatedly treated i.g. with *M. vaccae* ATCC 15483^T or its vehicle (BBS) before mating on days -21, -14, -7. Subsequently, their male and female offspring were subjected either to chronic adult stress (i.e., 21-days CSC in males and 7-weeks SIP in females; aim 1 – Schiele et al. (2026)) or to early life adversity (i.e., prenatal stress, consisting of RST alternating with SIP for 13 consecutive days in pregnant dams; aim 2 – Schiele et al. (2025)). The findings of my work provide novel insights into the intergenerational potential of "Old Friends"-based interventions to modulate behavioral, physiological, immunological and gut microbial alterations typically associated with stress in male offspring (Schiele et al., 2025; Schiele et al., 2026).

These results are discussed in the context of the psychoneuroimmunology literature and the "Old Friends" hypothesis, which emphasizes the role of immunoregulatory microorganisms in promoting stress resilience. Notably, the present findings align with previous evidence showing that maternal prebiotic and probiotic supplementation during pregnancy can beneficially influence offspring immunity, behavior and stress vulnerability (Hadizadeh et al., 2019; Hebert et al., 2021; Ratsika et al., 2021; Smith et al., 2020; Zhong et al., 2023).

Preconceptional maternal treatment with *M. vaccae* ATCC 15483^T enhances stress resilience and microbiome diversity in adult male offspring

To assess the intergenerational effects of *M. vaccae* ATCC 15483^T, male and female offspring were examined for sex-specific behavioral, physiological and immunological responses to CAS (i.e., 21-days CSC vs. SHC in males and 7-weeks SIP vs. GHC in females; Experiment 1). All experimental animals were euthanized in the morning following the last day of CAS exposure. In order to analyze the intergenerational effects of i.g. *M. vaccae* ATCC 15483^T administrations on the fecal microbiome composition, fecal pellets from BBS- and *M. vaccae* ATCC 15483^T-treated dams at various time points, including mating, birth and weaning, and from respective male offspring of Experiment 1 before and after sex-specific CAS exposure were collected (Schiele et al., 2026).

In male offspring, the CSC paradigm was used as a preclinical murine model for PTSD. This paradigm is well-established in the field of psychoneuroimmunology (Amoroso et al., 2020a; Amoroso et al., 2019; Amoroso et al., 2020b; Foertsch et al., 2017a; Foertsch et al., 2019; Kempter et al., 2021; Kempter et al., 2023; Langgartner et al., 2023a; Langgartner et al., 2015; Mazzari et al., 2023; Reber et al., 2016a; Reber et al., 2016b), and the results of my PhD experiment reproduced the characteristic CSC-induced outcomes (Schiele et al., 2026). Exposure to a dominant, aggressive resident induced general anxiety-related behavior (Amoroso et al., 2020a) in subordinate male mice represented by significant less entries into the contact zone during the NO condition (Schiele et al., 2026). On a physiological level, CSC resulted in adrenal hypertrophy and thymus involution (Langgartner et al., 2018a; Schiele et al., 2026), as well as bite wound-dependent splenomegaly (Foertsch et al., 2017a; Foertsch and Reber, 2020; Schiele et al., 2026), and reduced long bone length (Foertsch et al., 2017b; Schiele et al., 2026). Immunologically, CSC was associated with splenic invasion of myeloid cell populations, such as monocyte/MO-MDSCs, neutrophils and PMN-MDSCs (Kempter et al., 2023; Schiele et al., 2026), as well as increased splenic protein expression of TLR2 and TLR4 (Kempter et al., 2023; Langgartner et al., 2023a; Schiele et al., 2026).

In addition, isolated splenocytes exhibited increased cell viability under basal and LPS-stimulation conditions in absence of CORT, along with mild GC resistance at 0.1 µM CORT, but not at 5 µM CORT (Foertsch et al., 2017a; Kempter et al., 2021; Kempter et al., 2023; Schiele et al., 2026). CSC further increased chemotactic activity of isolated plasma WBCs (Kempter et al., 2023; Schiele et al., 2026), and reduced splenic $CD4^+CD25^+FoxP3^+$ Treg counts (Langgartner et al., 2023a; Schiele et al., 2026). Although CSC did not impair the suppression capacity of $CD4^+CD25^+$ Tregs on CFSE (carboxyfluorescein succinimidyl ester)-labeled splenocytes following CD3/ 28-stimulation, the calculated "Treg suppression capacity per animal" was reduced in CSC males of BBS-treated dams. This effect might be attributable to the observed reduction in splenic $FoxP3^+$ Treg numbers (Schiele et al., 2026). All the readouts described above were either ameliorated or completely prevented by the preconceptional administration of *M. vaccae* ATCC 15483^T to their respective dams, with the exception of the increased percentages of myeloid cell subpopulations in the spleen and enhanced chemotactic activity of WBCs isolated from the plasma (Schiele et al., 2026). Thus, in addition to the heat-inactivated saprophytic *M. vaccae* NCTC 11659 (Amoroso et al., 2020a; Amoroso et al., 2019; Langgartner et al., 2023a; Mazzari et al., 2023; Reber et al., 2016b), the facilitation of resilience to CSC exposure can be extended to other RGMs strains, including *M. vaccae* ATCC 15483^T.

Furthermore, consistent with previous findings using *M.vaccae* NCTC 11659 (Amoroso et al., 2020a; Desmond et al., 2023; Langgartner et al., 2023a; Reber et al., 2016b), *M. vaccae* ATCC 15483^T is showing an "adjuvant-like", or priming, effect on the immune system. This is reflected in SHC males by increased cell viability of splenocytes following *ex vivo* LPS stimulation, as well as by a reduced calculated "Treg suppression capacity per animal" (Schiele et al., 2026).

In female mice, the SIP was used as a validated model of chronic psychosocial stress, characterized by increased anxiety-like behavior, adrenal hypertrophy, reduced thymus weight, elevated plasma CORT levels, and impaired adult hippocampal neurogenesis (Schmidt et al., 2010; Yohn et al., 2019). In the present study, the SIP induced hyperactivity during the OF exploration in own-bred females born to BBS-treated mothers, as well as increased splenic monocyte/MO-MDSCs counts, independent of maternal *M. vaccae* ATCC 15483^T treatment (Schiele et al., 2026). While the increased splenic invasion of monocyte/MO-MDSCs was insensitive to *M. vaccae* ATCC 15483^T treatment (Mazzari et al., 2023), SIP-induced hyperactivity was prevented in an intergenerational manner by maternal *M. vaccae* ATCC 15483^T treatment (Schiele et al., 2026).

Taken together, these findings suggest that preconceptional administration of a non-pathogenic RGM confers protection against stress-induced behavioral alterations, independent of sex.

In line with human studies demonstrating that an increased α-diversity is associated with improved health outcomes (McBurney et al., 2019; Spragge et al., 2023; Walker et al., 2021), and that higher microbiome α-diversity can result from prebiotic dietary supplementation (Bester et al., 2023; Tandon et al., 2019), microbial analysis prior to CSC exposure in the present study revealed significant differences in both β- and α-diversity between male offspring of dams treated preconceptionally with *M. vaccae* ATCC 15483^T and those born to BBS-treated dams (Schiele et al., 2026). These differences included measures of richness, such as observed ASVs, the Shannon index, and the Chao1 index, but not Pielou's evenness (Schiele et al., 2026).

Supporting the hypothesis that the fecal microbiome contributes to the intergenerational effects mediated by *M. vaccae* ATCC 15483^T, observed ASVs and Choa1 index were negatively associated with physiological and immunological parameters that were increased by CSC in males born to BBS-treated dams. Conversely, these α-diversity measures were positively associated with parameters reduced by CSC in the same group (Schiele et al., 2026). Further analysis of fecal microbial community composition revealed an increased relative abundance of potent SCFA-producing bacterial taxa, such as Oscillospirales (Gophna et al., 2017), Clostridia vadin BB60 group (Wang et al., 2024a), and *Roseburia* (Abdugheni et al., 2022; Eeckhaut et al., 2013; Faden, 2022), in male offspring of dams treated with *M. vaccae* ATCC 15483^T (Schiele et al., 2026). These taxa are associated with beneficial health effects, as SCFAs, such as butyrate, contribute to immunoregulation through the induction of FoxP3$^+$ Tregs (Arpaia et al., 2013; Cushing et al., 2015; Dalile et al., 2019; Furusawa et al., 2013; Munyoki et al., 2024; Smith et al., 2013; Van den Bergh et al., 2020). Mechanistically, butyrate enhances acetylation of the *FoxP3* locus by inhibiting histone H3 deacetylases (HDACs), thereby promoting Treg differentiation (Arpaia et al., 2013). In line with the latter, the relative abundance of the identified SCFA-producing taxa positively correlated with Treg-related parameters, including splenic FoxP3$^+$ Treg counts and the calculated Treg suppression capacity per animal, in the present study (Schiele et al., 2026).

Additionally, *M. vaccae* ATCC 15483^T intergenerationally increased the relative abundance of pathological or inflammation-associated bacterial taxa (i.e., Desulfobacterota; Desulfovibrionales/ Desulfovibrionaceae), while reducing beneficial gut commensals or taxa with probiotic properties (Schiele et al., 2026)(i.e., Bacteroidota/ Bacteroidales/ Bacteroidaceae

and Muribaculaceae/ *Muribaculum*; Lactobacillales/ Lactobacillaceae/ *Lactobacillus*; *Lachnospiraceae bacterium COE1*) (Figliuolo et al., 2017; Gryaznova et al., 2022; Round and Mazmanian, 2009; Savino et al., 2018a; Savino et al., 2018b; Voigt et al., 2022; Wexler, 2007). These alterations in the gut microbiome might explain the adjuvant-like effects observed in SHC males born to *M. vaccae* ATCC 15483^T-treated dams(Schiele et al., 2026).

It should be noted that the fecal microbial analysis was conducted exclusively in male offspring of Experiment 1, as the key findings of maternal *M. vaccae* ATCC 15483^T treatment were primarily detected in CSC-exposed males, and not in females exposed to SIP (Schiele et al., 2026).

Since chronic stress is associated with gut dysbiosis, altered microbial community structure, and reduced α-diversity (Gautam et al., 2018; Khan et al., 2025; Reber et al., 2016a), the fecal microbiome was also analyzed after CSC exposure in the present study (Schiele et al., 2026). Notably, both β- and α-diversity, as well as the relative abundance of a SCFA-producing taxon, namely Ruminococcaceae (Gophna et al., 2017; Lee et al., 2020; Nogal et al., 2021), remained significantly different between *M. vaccae* ATCC 15483^T-CSC males and BBS-CSC males (Schiele et al., 2026). These findings strengthen the idea that immunoregulatory RGMs mediate intergenerational stress-protecting effects *via* the modulation of the microbiome. This interpretation is further supported by correlation analyses showing that α-diversity measures remained negatively associated with physiological and immunological parameters increased by CSC in males of BBS-treated dams, and positively associated with parameters reduced by CSC in the same group (Schiele et al., 2026). Moreover, consistent with evidence that SCFA-producing bacteria, particularly butyrate producers, promote FoxP3$^+$ Treg differentiation (Eeckhaut et al., 2013; Marteau, 2013), the relative abundance of Ruminococcaceae was positively correlated with Treg-related immunological parameters (Schiele et al., 2026). Considering the increased relative abundance of inflammation-associated taxa and the reduction in beneficial commensals or probiotic bacteria before CSC, these effects were no longer present following CSC exposure. Accordingly, the intergenerational effects of *M. vaccae* ATCC 15483^T on bacterial taxa associated with the adjuvant-like effects of *M. vaccae* ATCC 15483^T appear to be lost under CSC conditions (Schiele et al., 2026). Taken together, the results of the microbiome analysis following CSC exposure suggest that *M. vaccae* ATCC 15483^T induces intergenerational, long-lasting, and stress-resistant alterations in the fecal microbiome (Schiele et al., 2026).

To elucidate the mechanisms underlying the protective effects of *M. vaccae* ATCC 15483^T in the next male generation, microbiome analyses of maternal fecal samples were conducted

(Schiele et al., 2026). These analyses revealed reduced observed ASVs and Chao1 index in *M. vaccae* ATCC 15483^T-treated dams compared with BBS controls, but increased Pielou's evenness from birth to weaning. These patterns contrasted with those observed in male offspring prior to CSC exposure (Schiele et al., 2026).

Furthermore, β-diversity differed significantly between the *M. vaccae* ATCC 15483^T and the BBS group at mating and weaning (Schiele et al., 2026). Given the episodic nature of microbial β-diversity differences before and after pregnancy, these alterations might be related to the induction of Tregs, as previous studies in adult male mice have demonstrated that *M. vaccae* NCTC 11659 positively modulates the Treg compartment (Langgartner et al., 2023a; Reber et al., 2016b). Accordingly, it could be that administration of *M. vaccae* ATCC 15483^T also induces changes in the maternal immune system, which in turn influence the fecal microbiome, given the well-established bidirectional relationship between these systems (Amato et al., 2024; De Palma et al., 2015; Förger and Villiger, 2020; Fuhler, 2020). However, it must be considered that during pregnancy, particularly in early gestation, the maternal immune system undergoes profound adaptations, including substantial reduction in T cells and an increase of DCregs and Tregs to support fetal tolerance and to maintain healthy pregnancy (Amato et al., 2024; Förger and Villiger, 2020; Fuhler, 2020). These immunological adaptations might explain why no differences in α- or β-diversity were found at the time of birth in *M. vaccae* ATCC 15483^T- and BBS-treated dams (Schiele et al., 2026). Therefore, future studies investigating the effects of repeated *M. vaccae* ATCC 15483^T administration on the immune system of dams during pregnancy are warranted to clarify whether these effects are directly mediated by changes in the gut microbiome composition.

Moreover, community composition analysis revealed that, only at the time point of weaning, the relative abundances of SCFA-producing family Butyricoccaceae and inflammation-associated genus *Desulfovibrio* were significantly reduced in *M. vaccae* ATCC 15483^T-treated mothers compared with BBS-treated controls (Schiele et al., 2026). These contrasting findings between dams and their male offspring allow only limited conclusions regarding the underlying mechanisms of intergenerational transmission.

One prevailing hypothesis is that maternal circulating SCFAs represent the primary source of fetal SCFAs levels transferred *via* the placenta (Qin et al., 2024). Unfortunately, it was not possible to collect fecal samples from male pups at the earliest postnatal time point due to uncertainty in sex determination, which would otherwise have allowed characterization of the initial fecal microbiome in male offspring. Moreover, shortly after birth, the neonatal immune system undergoes substantial adaptations in response to environmental exposures,

among these are changes in nutritional intake (Jones et al., 2010). Therefore, the immunological or microbial composition of breast milk might also be influenced by *M. vaccae* ATCC 15483^{T} treatment and represents an additional factor of interest.

In support of the latter, both human (Bergström et al., 2014) and murine (Otaru et al., 2024) studies have shown that the cessation of breastfeeding leads to alterations in gut microbial parameters, including richness, evenness, community structure and composition. However, breast milk samples were not collected from the experimental dams in the present study (Schiele et al., 2026). Instead, mammary gland tissue from *M. vaccae* ATCC 15483^{T}- and BBS-treated mothers (Experiment 2) was analyzed for complement component C3 protein expression in collaboration with the Institute of Clinical and Experimental Trauma-Immunology (Ulm University Medical Center), but no significant differences were found between groups (Schiele et al., 2026).

Notably, Ramanan et al. (2020) showed that key intestinal immune traits, including colonic retinoacid-related orphan receptor gamma t (RORγt) expressing Tregs and IgA^{+} plasma cells, can be transmitted across multiple generations *via* non-genetic mechanisms in C57BL/6N mice. These effects are established during a critical early postnatal window and are mediated, at least in part, by maternal IgA levels transferred through breast milk, which shapes neonatal microbial colonization and further immune development (Ramanan et al., 2020).

Following the nursing phase, prior to weaning, offspring begin to consume solid food and engage coprophagia to enhance nutrient absorption and to facilitate microbial exchange among conspecifics (Murofushi et al., 2018; Robertson et al., 2019). Based on the hypothesis that maternal SCFA levels determine fetal SCFA concentrations (Qin et al., 2024), and on evidence that SCFA supplementation reduces the abundance of corresponding SCFA-producing bacteria in fecal cultures (Peterson et al., 2022), it is conceivable that reduced fetal SCFA levels might promote compensatory colonization by SCFA-producing taxa in the offspring. Therefore, one potential transmission mechanism underlying the intergenerational protective effects of *M. vaccae* ATCC 15483^{T} might involve the transfer of reduced levels of SCFA-producing bacteria from mothers to offspring *via* coprophagia, followed by compensatory expansion of these taxa, suggesting a currently undefined negative feedback mechanism (Schiele et al., 2026).

Supporting this approach, Qing et al. (2025) emphasized the importance of postnatal maternal-infant microbial transmission for the beneficial effects of prenatal probiotic interventions. Building on early life priming, the induction of FoxP3^{+}RORγt^{+} Tregs in the intestine

of offspring occurs during the transition to solid food, a developmental phase characterized by increased SCFA availability and long-term immune printing (Al Nabhani et al., 2019). Together, these findings suggest that early postnatal maternal influences and subsequent microbiome-driven processes during weaning represent sequential windows of opportunity for shaping long-term immune outcomes.
In this context, cross-fostering and co-housing experiments might help to disentangle the relative contribution and timing of microbial and metabolite transfer. Should future studies confirm that the intergenerational stress-protective effects of *M. vaccae* ATCC 15483^{T} are mediated by postnatal microbial transmission, this would have important translational implications. In humans, the exposure to maternal (fecal) microbiota during natural birth as well as through breastfeeding and skin contact contribute to the establishment of the pioneer microbiome and healthy development of the newborn (Bogaert et al., 2023; Fasano et al., 2024).

In the present study, the CAS paradigms employed, CSC in males and SIP in females, were sex-specific and therefore did not permit direct comparison between male and female offspring, which represents a key limitation (Schiele et al., 2026). A potentially more suitable approach for such comparisons would be the use of the repeated social defeat stress (RSDS) paradigm (Takahashi et al., 2017). However, the RSDS model also presents challenges, as experimental females are typically exposed to a male aggressor, whereas males encounter same-sex opponents. Nevertheless, it might be that outcomes dependent on physical confrontation, such as splenomegaly and GC resistance (Foertsch et al., 2017a; Foertsch and Reber, 2020; Kempter et al., 2023; Schiele et al., 2024) might also be observed in females subjected to RSDS. Moreover, stress paradigms based on repeated or chronic restraint stress (Avitsur et al., 2011; Sheridan et al., 2000), as well as chronic mild stressor exposure (Sachs et al., 2014) represent additional options applicable to both sexes. However, the absence of the social component, which is the interaction of conspecifics, should be considered when interpreting findings related to chronic psychosocial stress.
An alternative approach enabling direct comparison between both sexes would be a modified version of the CSC paradigm combining sensory contact exposure to stressed conspecifics with standardized sterile intraperitoneal wounding (Schiele et al., 2024).

This adapted paradigm induced characteristic chronic stress-related physiological and immunological features, including adrenal hypertrophy and mild splenic GC resistance, while avoiding uncontrolled bite injuries inherent to the CSC model. Importantly, stress-induced

GC resistance in this model appears to depend on the interaction between witnessing psychosocial stress and artificial physical injury (Foertsch et al., 2017a; Foertsch et al., 2020; Kempter et al., 2023). Due to its reliance on sensory stress exposure and individual housing rather than direct aggression, this approach could be applicable to female mice, thereby facilitating sex-comparative analyses of chronic psychosocial stress effects.

In summary, the present findings from Experiments 1 and 2 (Schiele et al., 2026), together with previous evidence on maternal probiotic and prebiotic supplementation during pregnancy (Hadizadeh et al., 2019; Hebert et al., 2021; Ratsika et al., 2021; Smith et al., 2020; Zhong et al., 2023), support the hypothesis that repeated preconceptional administration of non-pathogenic RGMs, such as *M. vaccae* ATCC 15483^{T}, enhances intergenerational stress resilience by promoting immunoregulatory mechanisms and diversifying the gut microbiome (Schiele et al., 2026).

Preconceptional *M. vaccae* ATCC 15483^{T} treatment promotes intergenerational resilience in prenatal stress-exposed male offspring

The prenatal period is particularly vulnerable to external influences such as stress (Clayborne et al., 2024; Clayborne et al., 2023; Davis and Narayan, 2020; Dhaliwal et al., 2023), and as outlined in the Introduction ("*Prenatal stress as driver of long-term health risks*"), prenatal stress is associated with adverse long-term outcomes in offspring.

To investigate whether repeated administration of *M. vaccae* ATCC 15483^{T} modulates the effects of PS, treated dams during pregnancy were subjected to a prenatal stress paradigm (Experiment 3) (Schiele et al., 2025). This model involved alternating restraint stress (twice daily for 1 h, with at least 3 h between sessions) and 24 h group-housing with unfamiliar females for 13 consecutive days (Hillerer et al., 2011). Following birth and weaning, adolescent male and female offspring were assessed for anxiety-related, (OF/NO, SPAT, EPM), social (SPAT), anhedonic- (SPT), and depressive-like (FST) behaviors (Schiele et al., 2025). These behavioral domains were selected based on robust evidence linking PS to various long-term behavioral alterations (Gur et al., 2017; Ma et al., 2024; Moura et al., 2022; Moura et al., 2020; Mueller and Bale, 2008; Zhang et al., 2021).

In addition, physiological, bone-related and immunological parameters were evaluated (Experiment 3; Experiment 1 in Schiele et al. (2025) study on prenatal stress and *M. vaccae* ATCC 15483^{T}). Interestingly, PS did not consistently affect behavioral outcomes and typical physiological stress markers, including adrenal, thymus and spleen weights, or basal and

post-acute restraint stress plasma CORT concentrations in either sex (Schiele et al., 2025). This contrasts with previous preclinical studies reporting increased plasma CORT levels following acute restraint stress (Barbazanges et al., 1996; Brunton and Russell, 2010). Female offspring appeared less susceptible to PS, consistent with studies indicating greater female vulnerability to postnatal stressors such as maternal separation (Gebauer et al., 2025; Mazzari et al., 2023; Schimmele et al., 2025).

Despite the limited behavioral and physiological effects, sex-specific intergenerational stress-protective effects of *M. vaccae* ATCC 15483^{T} were observed at the immunological level in male offspring (Schiele et al., 2025). PS induced persistent systemic immune activation, reflected by elevated splenic IgG expression in adult males, which was prevented in offspring of *M. vaccae* ATCC 15483^{T}-treated dams. [The IgG expression data of males in Experiment 4; cohort 2 in Schiele et al. (2025), were included in a doctoral thesis of a medical student I supervised during my PhD: Ernst, 2023 – details regarding contributions can be found in the co-author list and acknowledgments of Schiele et al. (2025)].

Elevated IgG levels are indicative for bacterial translocation, originating from pathogenic (Vazquez-Torres et al., 1999; Vujkovic-Cvijin et al., 2022) or commensal sources (Rissing et al., 1979; Zeng et al., 2016), and are consistent with results showing that pre- and postnatal stress impair intestinal barrier integrity (García-Ródenas et al., 2006; Leigh et al., 2023). Notably, IgG antibodies predominantly target antigens of gram-negative commensals, such as murine lipoprotein, thereby contributing to the host defense against systemic infections (Zeng et al., 2016), suggesting that the observed increase in IgG might reflect a compensatory immune response to microbial translocation.

Supporting the latter, plasma levels of LBP, a marker for long-term LPS exposure (Voigt et al., 2022), were reduced in PS males (Schiele et al., 2025), likely reflecting increased phagocytotic clearance of IgG-opsonized bacteria (Bournazos et al., 2016). Importantly, elevated IgG expression was not detectable at birth (Experiment 5) (Schiele et al., 2025), indicating that these effects were not maternally transferred prenatally or *via* lactation (Xu et al., 2024). [The IgG protein expression data from male pups in Experiment 5; cohort 3 in Schiele et al. (2025), were part of another doctoral thesis of a medical student I supervised during my PhD: Blank, 2024 – details regarding contributions can be found in the co-author list and acknowledgments of Schiele et al. (2025)].

Consistent with this immune activation profile, PS-exposed males exhibited increased chemotactic activity of WBCs, as well as enhanced viability of isolated splenic cells following *ex vivo* LPS stimulation (Schiele et al., 2025). These findings parallel observations using the

CSC model (Kempter et al., 2023) and are consistent with previous studies using the RSDS paradigm demonstrating microbiota-dependent immune priming under chronic stress conditions (Allen et al., 2012; Bailey et al., 2007).

Similarly, preconceptional *M. vaccae* ATCC 15483^T treatment prevented the PS-induced increase in chemotactic activity and induced immune-priming effects in non-stressed offspring (Schiele et al., 2025), mirroring findings in CSC-exposed males and previous studies (Langgartner et al., 2023a; Loupy et al., 2019; Lowry et al., 2007; Mazzari et al., 2023; Reber et al., 2016b).

In addition to the immunological alterations, PS induced an osteopenic/osteoporotic phenotype in male offspring (Experiment 3), which was absent in males subjected to single-housing for additional 28 days (Experiment 4; Experiment 2 in the study of Schiele et al. (2025) on prenatal stress and *M. vaccae* ATCC 15483^T). These findings raise the question of whether multiple mild acute stressors are required to induce impaired bone metabolism, or whether the observed phenotype is transient (Schiele et al., 2025).

Previous work using the CSC model has shown that chronic low-grade inflammation, particularly mediated by myeloid immune cells, impairs bone metabolism and fracture healing (Foertsch et al., 2017b; Haffner-Luntzer et al., 2019; Tschaffon-Müller et al., 2023). In line with these findings, *M. vaccae* ATCC 15483^T ameliorated stress-induced bone deficits in both CSC- (Experiment 1) and PS-exposed male offspring (Experiment 3), although underlying mechanisms remain unclear (Schiele et al., 2025). Notably, in contrast to male-specific effects of prenatal and adult stress, postnatal stress models such as maternal separation predominantly affect females, a vulnerability that was also mitigated by *M. vaccae* NCTC 11659 (Gebauer et al., 2025; Schimmele et al., 2025).

A key limitation of the present PS study is the multifactorial design, which complicates the attribution of observed effects to specific stressors (Schiele et al., 2025). Future studies should aim to isolate the effects of PS by minimizing additional postnatal stress exposure and assessing outcomes at defined developmental time points, such as shortly after weaning or at selected later life stages. This approach would help to clarify the specific contribution of PS to the development of a compromised bone phenotype.

Finally, the role of the microbiome-gut-bone axis definitively warrants further investigation (Zaiss et al., 2019). Chronic immune activation induced by PS might impair intestinal barrier function and promote bacterial translocation, thereby contributing to altered bone metabolism (Schiele et al., 2025). However, the mechanism by which *M. vaccae* ATCC 15483^T confer intergenerational stress resilience remain to be fully elucidated.

Conclusions

During my PhD studies, I primarily focused on a research project investigating the intergenerational stress-protective effects of the immunoregulatory saprophytic microbe *M. vaccae* ATCC 15483^T. This project was conducted in close collaboration with Prof. David A. Slattery and his PhD student Pei-Ling Tsai from the Goethe University Frankfurt. The results demonstrated that three repeated i.g. administrations of *M. vaccae* ATCC 15483^T to nulliparous C57BL/6N female mice prior to mating protected mainly their male offspring against the negative consequences of chronic psychosocial stress experienced either during adulthood (Experiment 1; Schiele et al. (2026)) or prenatally (Experiments 3 and 4; Schiele et al. (2025)).

This research builds on an extensive body of work on the rapid-growing mycobacterium strain *M. vaccae* NCTC 11659, including studies on its various administration routes and its applications across diverse experimental contexts (Amoroso et al., 2020a; Amoroso et al., 2019; Amoroso et al., 2020b; Amoroso et al., 2021; Bowers et al., 2020; Desmond et al., 2023; Fonken et al., 2018a; Foxx et al., 2020; Frank et al., 2018a; Gebauer et al., 2025; Hassell et al., 2019; Langgartner et al., 2023a; Loupy et al., 2019; Reber et al., 2016b; Sanchez et al., 2022; Schimmele et al., 2025). In addition, other "Old Friends", such as *M. aurum* DSM 33539, have been shown to enhance stress resilience (Langgartner et al., 2024). Given the feasibility of cultivating *M. vaccae* ATCC 15483^T in-house and accumulating evidence of its stress-protective and antiinflammatory properties (Desmond et al., 2024; Loupy et al., 2021), this strain was selected for preconceptional administration *via* the less invasive i.g. route.

In Experiment 1, the well-established CSC paradigm was employed in adult male offspring. The characteristic behavioral, physiological, and immunological effects of chronic psychosocial stress were successfully replicated (Amoroso et al., 2020a; Foertsch et al., 2017b; Kempter et al., 2021; Kempter et al., 2023; Langgartner et al., 2023a; Langgartner et al., 2018a; Langgartner et al., 2015; Reber et al., 2016a), and most of these CSC-induced alterations were either prevented or attenuated in an intergenerational manner by maternal *M. vaccae* ATCC 15483^T treatment (Schiele et al., 2026). In female offspring, the SIP model was employed due to sex-specific constraints of stress models, instead of the CSC paradigm (Mazzari et al., 2023; Schmidt et al., 2010; Yohn et al., 2019). Although the SIP did not significantly affect physiological and immunological parameters, it induced hyperactivity in offspring of BBS-treated dams, which was absent in females born to *M. vaccae* ATCC 15483^T-treated mothers (Schiele et al., 2026).

Importantly, as male and female offspring were never directly exposed to the immunoregulatory agent, these findings demonstrate true intergenerational effects (Klengel et al., 2016). Microbiome analyses further revealed that male offspring of *M. vaccae* ATCC 15483^{T}-treated dams exhibited increased gut microbial diversity and enrichment of SCFA-producing taxa, supporting a role for microbiome-mediated immunoregulation in promoting stress resilience, particularly via Treg induction (Schiele et al., 2026).

Using a PS paradigm (Hillerer et al., 2011) combining restraint stress and the SIP, it was further demonstrated that early life adversity exerts sex-specific effects on behavior, physiology and immune function (Schiele et al., 2025). In male offspring, PS induced age-dependent anxiogenic effects and transient impairments in bone metabolism. PS was also associated with immune activation, reflected by increased IgG levels and reduced plasma LBP concentrations, suggesting enhanced IgG-mediated clearance of translocated bacterial antigens (Bournazos et al., 2016; Zeng et al., 2016). Notably, these effects were prevented in male offspring of *M. vaccae* ATCC 15483^{T}-treated dams, indicating a protective intergenerational effect (Schiele et al., 2025).

In addition to my main PhD project, I contributed to the development of a controlled experimental model to investigate stress-induced *ex vivo* functional splenic GC resistance (data not shown in this thesis; Schiele et al. (2024)). Building on findings from the CSC paradigm showing that both aggressive encounters and surgical wounding can induce GC resistance (Foertsch et al., 2017a; Foertsch et al., 2020; Foertsch and Reber, 2020; Kempter et al., 2021; Kempter et al., 2023), a modified model combining sterile intraperitoneal incision one week prior to stress exposure with individual housing in sensory contact to physically stressed conspecifics was established (Schiele et al., 2024). This approach enabled the induction of mild functional GC resistance in splenic myeloid cells under standardized conditions, thereby providing a valuable tool for mechanistic studies. Moreover, due to its reliance on sensory contact stress exposure and individual housing rather than direct physical confrontation, this model might also be applicable to female mice, facilitating the investigation of sex-specific effects of chronic psychosocial stress.

Overall, the work presented in this thesis provides a major advancement:

It extends the "Old Friends" hypothesis by demonstrating that preconceptional administration of non-pathogenic *M. vaccae* ATCC 15483^{T} to female C57BL/6N mice promotes intergenerational resilience to both chronic adult stress (**aim 1**) and prenatal stress (**aim 2**) in offspring, with more pronounced effects in males. These findings highlight the importance of maternal microbial exposure in shaping long-term stress resilience and encourage future studies to elucidate the underlying mother-to-offspring transmission mechanisms.

Finally, the findings support a novel and potentially translatable strategy to enhance stress resilience across generations through repeated administration of heat-inactivated immunoregulatory RGMs. Given the increasing prevalence of stress-related psychiatric diseases, particularly in industrialized societies characterized by reduced microbial diversity and impaired immunoregulation, such microbiome-targeted interventions might represent a promising avenue for prevention and therapy.

Summary

Chronic psychosocial stress is a well-established risk factor for the development of somatic and psychiatric disorders, such as inflammatory bowel disease (IBD), depression and post-traumatic stress disorder (PTSD) (Agid et al., 1999; de Kloet et al., 2005; Gola et al., 2013; Lindqvist et al., 2014; Pace et al., 2006; Podolsky, 2002; Reber, 2012; Reber et al., 2016a; Robertson et al., 1989; Rohleder, 2014). It is associated with chronic low-grade inflammation, immune dysregulation, but also glucocorticoid (GC) insensitivity of myeloid immune cells (Amoroso et al., 2020b; Kempter et al., 2023; Miller and Raison, 2016; Raison and Miller, 2003).

The "Old Friends" hypothesis proposes that exposure to co-evolved immunoregulatory microorganisms, more prevalent in rural than urban environments, can protect against inflammatory and stress-related diseases (Langgartner et al., 2019). Importantly, both the presence and timing of microbial exposure are critical. Maternal exposure to such microorganisms has been associated with improved health outcomes in offspring, as evidenced in epidemiological studies (Ahlgren et al., 2012; Anumula et al., 2024; Eldeirawi et al., 2009; Hou et al., 2009; Koren et al., 2024; Li et al., 2011; Pereg et al., 2008; Riedler et al., 2001; Rottem et al., 2005; Söderström et al., 2012). Preclinical studies further indicate that administration of non-pathogenic rapid-growing mycobacteria (RGMs) promotes immunoregulation via regulatory T cells (Tregs) and antiinflammatory cytokine production (Amoroso et al., 2021).

In this thesis, I investigated the intergenerational stress-protective effects of the heat-inactivated, saprophytic "Old Friend" *M. vaccae* ATCC 15483^{T}, administered intragastrically to nulliparous female C57BL/6N mice prior to mating. Their male and female offspring were subsequently exposed to chronic psychosocial stress during adulthood, using the CSC paradigm and the SIP, respectively (Schiele et al., 2026).

Across behavioral, physiological and immunological parameters, preconceptional *M. vaccae* ATCC 15483^{T} treatment enhanced stress resilience, especially in the adult male offspring. Moreover, microbial analyses revealed an increased α-diversity and a shift in microbial community composition in male offspring of *M. vaccae* ATCC 15483^{T}-treated dams, including the enrichment of short-chain fatty acid (SCFA)-producing taxa, which are associated with beneficial immunoregulation and Treg induction (Schiele et al., 2026).

Given the vulnerability of the prenatal period to internal and external stressors (Clayborne et al., 2024; Clayborne et al., 2023; Dhaliwal et al., 2023), I further investigated whether maternal *M. vaccae* ATCC 15483^T treatment protects male and female offspring from the negative consequences of prenatal stress (PS) (Schiele et al., 2025). While female offspring showed no clear stress-related phenotype, PS-exposed male offspring exhibited prolonged immune activation, reflected by increased IgG levels and reduced plasma lipopolysaccharide-binding protein (LBP) concentrations, suggesting an impaired intestinal barrier integrity and increased bacterial translocation. In addition, a transient, male-specific osteopenic/osteoporotic bone phenotype indicated disruption of bone metabolism. Notably, these PS-induced alterations were absent in male offspring of *M. vaccae* ATCC 15483^T-treated dams (Schiele et al., 2025). These findings highlight pronounced sex-specific effects and raise important questions regarding the mechanisms underlying mother-to-offspring transmission of stress resilience.

In a complementary side project (data not shown in this thesis, Schiele et al. (2024)), a modified CSC-based sensory contact stress model was developed to investigate stress-induced GC resistance under controlled conditions. This approach enables the study of immune-related stress responses without uncontrolled physical injury and provides the possibility to expose both male and female mice to comparable stress conditions, thereby facilitating direct sex-based comparisons.

In summary, the findings of this thesis extent the “Old Friends” hypothesis by showing that preconceptional administration of *M. vaccae* ATCC 15483^T promotes intergenerational stress resilience in a sex-specific manner. These results provide a foundation for future studies investigating the mechanisms of maternal microbial programming and highlight the potential of microbiome-targeted interventions as preventive strategies for stress-associated somatic and mental disorders.

List of References

Abbot, N.C., Beck, J.S., Feval, F., Weiss, F., Mobayen, M.H., Ghazi-Saidi, K., Dowlati, Y., Velayati, A.A., Stanford, J.L., 2002. Immunotherapy with Mycobacterium vaccae and peripheral blood flow in long-treated leprosy patients, a randomised, placebo-controlled trial. Eur J Vasc Endovasc Surg 24, 202-208.

Abdugheni, R., Wang, W.Z., Wang, Y.J., Du, M.X., Liu, F.L., Zhou, N., Jiang, C.Y., Wang, C.Y., Wu, L., Ma, J., Liu, C., Liu, S.J., 2022. Metabolite profiling of human-originated Lachnospiraceae at the strain level. Imeta 1, e58.

Agid, O., Shapira, B., Zislin, J., Ritsner, M., Hanin, B., Murad, H., Troudart, T., Bloch, M., Heresco-Levy, U., Lerer, B., 1999. Environment and vulnerability to major psychiatric illness: a case control study of early parental loss in major depression, bipolar disorder and schizophrenia. Mol Psychiatry 4, 163-172.

Ahlgren, C., Odén, A., Lycke, J., 2012. A nationwide survey of the prevalence of multiple sclerosis in immigrant populations of Sweden. Mult Scler 18, 1099-1107.

Al Nabhani, Z., Dulauroy, S., Marques, R., Cousu, C., Al Bounny, S., Déjardin, F., Sparwasser, T., Bérard, M., Cerf-Bensussan, N., Eberl, G., 2019. A Weaning Reaction to Microbiota Is Required for Resistance to Immunopathologies in the Adult. Immunity 50, 1276-1288.e1275.

Allen, R.G., Lafuse, W.P., Galley, J.D., Ali, M.M., Ahmer, B.M., Bailey, M.T., 2012. The intestinal microbiota are necessary for stressor-induced enhancement of splenic macrophage microbicidal activity. Brain Behav Immun 26, 371-382.

Almansa, C., Rey, E., Sánchez, R.G., Sánchez, A.A., Díaz-Rubio, M., 2009. Prevalence of functional gastrointestinal disorders in patients with fibromyalgia and the role of psychologic distress. Clin Gastroenterol Hepatol 7, 438-445.

Alonso, J., Petukhova, M., Vilagut, G., Chatterji, S., Heeringa, S., Üstün, T.B., Alhamzawi, A.O., Viana, M.C., Angermeyer, M., Bromet, E., Bruffaerts, R., de Girolamo, G., Florescu, S., Gureje, O., Haro, J.M., Hinkov, H., Hu, C.Y., Karam, E.G., Kovess, V., Levinson, D., Medina-Mora, M.E., Nakamura, Y., Ormel, J., Posada-Villa, J., Sagar, R., Scott, K.M., Tsang, A., Williams, D.R., Kessler, R.C., 2011. Days out of role due to common physical and mental conditions: results from the WHO World Mental Health surveys. Mol Psychiatry 16, 1234-1246.

Amato, K.R., Pradhan, P., Mallott, E.K., Shirola, W., Lu, A., 2024. Host-gut microbiota interactions during pregnancy. Evolution, Medicine, and Public Health 12, 7-23.

Amoroso, M., Bottcher, A., Lowry, C.A., Langgartner, D., Reber, S.O., 2020a. Subcutaneous Mycobacterium vaccae promotes resilience in a mouse model of chronic psychosocial stress when administered prior to or during psychosocial stress. Brain Behav Immun 87, 309-317.

Amoroso, M., Kempter, E., Eleslambouly, T., Lowry, C.A., Langgartner, D., Reber, S.O., 2019. Intranasal Mycobacterium vaccae administration prevents stress-induced aggravation of dextran sulfate sodium (DSS) colitis. Brain Behav Immun 80, 595-604.

Amoroso, M., Kempter, E., Langgartner, D., Gross, P., Reber, S.O., 2020b. Inducing a stressed phenotype in healthy recipient mice by adoptively transferring CD4(+) lymphocytes from mice undergoing chronic psychosocial stress. Psychoneuroendocrinology 122, 104898.

Amoroso, M., Langgartner, D., Lowry, C.A., Reber, S.O., 2021. Rapidly Growing Mycobacterium Species: The Long and Winding Road from Tuberculosis Vaccines to Potent Stress-Resilience Agents. Int J Mol Sci 22.

Antonson, A.M., Evans, M.V., Galley, J.D., Chen, H.J., Rajasekera, T.A., Lammers, S.M., Hale, V.L., Bailey, M.T., Gur, T.L., 2020. Unique maternal immune and functional microbial profiles during prenatal stress. Sci Rep 10, 20288.

Anumula, S., Nalla, K., Pandala, P., Kotha, R., Sr., Harsha, N., 2024. Rural Versus Urban Mothers' Microbiome Difference and Its Effect on Neonates: A Systematic Review. Cureus 16, e55607.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., Rudensky, A.Y., 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 504, 451-455.

Avitsur, R., Mays, J.W., Sheridan, J.F., 2011. Sex differences in the response to influenza virus infection: modulation by stress. Horm Behav 59, 257-264.

Avitsur, R., Padgett, D.A., Dhabhar, F.S., Stark, J.L., Kramer, K.A., Engler, H., Sheridan, J.F., 2003. Expression of glucocorticoid resistance following social stress requires a second signal. J Leukoc Biol 74, 507-513.

Azuma, K., Ogura, M., Kondo, H., Suzuki, A., Hayashi, S., Iinuma, M., Onozuka, M., Kubo, K.Y., 2017. Maternal Active Mastication during Prenatal Stress Ameliorates Prenatal Stress-Induced Lower Bone Mass in Adult Mouse Offspring. Int J Med Sci 14, 348-355.

Babu, S., Blauvelt, C.P., Kumaraswami, V., Nutman, T.B., 2006. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. J Immunol 176, 3248-3256.

Bach, J.F., 2002. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 347, 911-920.

Bai, F., Li, X., Clay, M., Lindstrom, T., Skolnick, P., 2001. Intra- and interstrain differences in models of "behavioral despair". Pharmacol Biochem Behav 70, 187-192.

Bailey, M.T., Engler, H., Powell, N.D., Padgett, D.A., Sheridan, J.F., 2007. Repeated social defeat increases the bactericidal activity of splenic macrophages through a Toll-like

receptor-dependent pathway. Am J Physiol Regul Integr Comp Physiol 293, R1180-1190.

Bale, T.L., 2016. The placenta and neurodevelopment: sex differences in prenatal vulnerability. Dialogues Clin Neurosci 18, 459-464.

Bangasser, D.A., Valentino, R.J., 2014. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. Front Neuroendocrinol 35, 303-319.

Baranyi, J., Bakos, N., Haller, J., 2005. Social instability in female rats: the relationship between stress-related and anxiety-like consequences. Physiol Behav 84, 511-518.

Barbazanges, A., Piazza, P.V., Le Moal, M., Maccari, S., 1996. Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. J Neurosci 16, 3943-3949.

Batt, S.M., Minnikin, D.E., Besra, G.S., 2020. The thick waxy coat of mycobacteria, a protective layer against antibiotics and the host's immune system. Biochem J 477, 1983-2006.

Bazzi, S., Modjtahedi, H., Mudan, S., Akle, C., Bahr, G.M., 2015. Analysis of the immunomodulatory properties of two heat-killed mycobacterial preparations in a human whole blood model. Immunobiology 220, 1293-1304.

Behan, A.T., van den Hove, D.L., Mueller, L., Jetten, M.J., Steinbusch, H.W., Cotter, D.R., Prickaerts, J., 2011. Evidence of female-specific glial deficits in the hippocampus in a mouse model of prenatal stress. Eur Neuropsychopharmacol 21, 71-79.

Benchimol, E.I., Mack, D.R., Guttmann, A., Nguyen, G.C., To, T., Mojaverian, N., Quach, P., Manuel, D.G., 2015. Inflammatory bowel disease in immigrants to Canada and their children: a population-based cohort study. Am J Gastroenterol 110, 553-563.

Benediktsson, R., Calder, A.A., Edwards, C.R., Seckl, J.R., 1997. Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clin Endocrinol (Oxf) 46, 161-166.

Berg-Hansen, P., Moen, S.M., Sandvik, L., Harbo, H.F., Bakken, I.J., Stoltenberg, C., Celius, E.G., 2015. Prevalence of multiple sclerosis among immigrants in Norway. Mult Scler 21, 695-702.

Bergström, A., Skov, T.H., Bahl, M.I., Roager, H.M., Christensen, L.B., Ejlerskov, K.T., Mølgaard, C., Michaelsen, K.F., Licht, T.R., 2014. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. Appl Environ Microbiol 80, 2889-2900.

Berthoud, H.R., Neuhuber, W.L., 2000. Functional and chemical anatomy of the afferent vagal system. Auton Neurosci 85, 1-17.

Bester, A., O'Brien, M., Cotter, P.D., Dam, S., Civai, C., 2023. Shotgun Metagenomic Sequencing Revealed the Prebiotic Potential of a Fruit Juice Drink with Fermentable Fibres in Healthy Humans. Foods 12.

Beurel, E., Toups, M., Nemeroff, C.B., 2020. The Bidirectional Relationship of Depression and Inflammation: Double Trouble. Neuron 107, 234-256.

Blaser, M.J., 2017. The theory of disappearing microbiota and the epidemics of chronic diseases. Nat Rev Immunol 17, 461-463.

Blaser, M.J., Falkow, S., 2009. What are the consequences of the disappearing human microbiota? Nat Rev Microbiol 7, 887-894.

Böbel, T.S., Hackl, S.B., Langgartner, D., Jarczok, M.N., Rohleder, N., Rook, G.A., Lowry, C.A., Gündel, H., Waller, C., Reber, S.O., 2018. Less immune activation following social stress in rural vs. urban participants raised with regular or no animal contact, respectively. Proc Natl Acad Sci U S A 115, 5259-5264.

Boenickse, R., Juhasz, E., 1964. [DESCRIPTION OF THE NEW SPECIES MYCOBACTERIUM VACCAE N. SP]. Zentralbl Bakteriol Orig 192, 133-135.

Bogaert, D., van Beveren, G.J., de Koff, E.M., Lusarreta Parga, P., Balcazar Lopez, C.E., Koppensteiner, L., Clerc, M., Hasrat, R., Arp, K., Chu, M., de Groot, P.C.M., Sanders, E.A.M., van Houten, M.A., de Steenhuijsen Piters, W.A.A., 2023. Mother-to-infant microbiota transmission and infant microbiota development across multiple body sites. Cell Host Microbe 31, 447-460.e446.

Borrelli, R., Brussino, L., Lo Sardo, L., Quinternetto, A., Vitali, I., Bagnasco, D., Boem, M., Corradi, F., Badiu, I., Negrini, S., Nicola, S., 2025. Sex-Based Differences in Asthma: Pathophysiology, Hormonal Influence, and Genetic Mechanisms. Int J Mol Sci 26.

Bournazos, S., Wang, T.T., Ravetch, J.V., 2016. The Role and Function of Fcγ Receptors on Myeloid Cells. Microbiol Spectr 4.

Bouxsein, M.L., Boyd, S.K., Christiansen, B.A., Guldberg, R.E., Jepsen, K.J., Müller, R., 2010. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. J Bone Miner Res 25, 1468-1486.

Bowers, S.J., Lambert, S., He, S., Lowry, C.A., Fleshner, M., Wright, K.P., Jr., Turek, F.W., Vitaterna, M.H., 2020. Immunization with a heat-killed bacterium, Mycobacterium vaccae NCTC 11659, prevents the development of cortical hyperarousal and a PTSD-like sleep phenotype after sleep disruption and acute stress in mice. Sleep.

Bowman, R.E., MacLusky, N.J., Sarmiento, Y., Frankfurt, M., Gordon, M., Luine, V.N., 2004. Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. Endocrinology 145, 3778-3787.

Breslau, J., Borges, G., Hagar, Y., Tancredi, D., Gilman, S., 2009. Immigration to the USA and risk for mood and anxiety disorders: variation by origin and age at immigration. Psychol Med 39, 1117-1127.

Breslau, J., Borges, G., Tancredi, D., Saito, N., Kravitz, R., Hinton, L., Vega, W., Medina-Mora, M.E., Aguilar-Gaxiola, S., 2011. Migration from Mexico to the United States

and subsequent risk for depressive and anxiety disorders: a cross-national study. Arch Gen Psychiatry 68, 428-433.

Breslau, N., 2009. The epidemiology of trauma, PTSD, and other posttrauma disorders. Trauma Violence Abuse 10, 198-210.

Bronson, S.L., Bale, T.L., 2014. Prenatal stress-induced increases in placental inflammation and offspring hyperactivity are male-specific and ameliorated by maternal antiinflammatory treatment. Endocrinology 155, 2635-2646.

Brunton, P.J., Russell, J.A., 2010. Prenatal social stress in the rat programmes neuroendocrine and behavioural responses to stress in the adult offspring: sex-specific effects. J Neuroendocrinol 22, 258-271.

Buchanan, T.W., Bagley, S.L., Stansfield, R.B., Preston, S.D., 2012. The empathic, physiological resonance of stress. Soc Neurosci 7, 191-201.

Buckley, T., Stannard, A., Bartrop, R., McKinley, S., Ward, C., Mihailidou, A.S., Morel-Kopp, M.C., Spinaze, M., Tofler, G., 2012. Effect of early bereavement on heart rate and heart rate variability. Am J Cardiol 110, 1378-1383.

Burich, A., Hershberg, R., Waggie, K., Zeng, W., Brabb, T., Westrich, G., Viney, J.L., Maggio-Price, L., 2001. Helicobacter-induced inflammatory bowel disease in IL-10- and T cell-deficient mice. Am J Physiol Gastrointest Liver Physiol 281, G764-778.

Busch, M., Chourbaji, S., Dammann, P., Gerold, S., Heamisch, A., Jirkof, P., Oehlert, P., Osterkamp, A., Ott, S., Peters, S., Spekl, K., Tsai, P.P., Reber, S.O., 2013. Ausschuss für tiergerechte Labortierhaltung zur Einzelhaltung von Mäusen zu Versuchszwecken. GV-SOLAS, 1-8.

Cabieses, B., Uphoff, E., Pinart, M., Antó, J.M., Wright, J., 2014. A systematic review on the development of asthma and allergic diseases in relation to international immigration: the leading role of the environment confirmed. PLoS One 9, e105347.

Calarge, C.A., Butcher, B.D., Burns, T.L., Coryell, W.H., Schlechte, J.A., Zemel, B.S., 2014. Major depressive disorder and bone mass in adolescents and young adults. J Bone Miner Res 29, 2230-2237.

Cámara, R.J., Gander, M.L., Begré, S., von Känel, R., 2011. Post-traumatic stress in Crohn's disease and its association with disease activity. Frontline Gastroenterol 2, 2-9.

Camporota, L., Corkhill, A., Long, H., Lordan, J., Stanciu, L., Tuckwell, N., Cross, A., Stanford, J.L., Rook, G.A., Holgate, S.T., Djukanovic, R., 2003. The effects of Mycobacterium vaccae on allergen-induced airway responses in atopic asthma. Eur Respir J 21, 287-293.

Cananzi, F.C., Mudan, S., Dunne, M., Belonwu, N., Dalgleish, A.G., 2013. Long-term survival and outcome of patients originally given Mycobacterium vaccae for metastatic malignant melanoma. Hum Vaccin Immunother 9, 2427-2433.

Carabotti, M., Scirocco, A., Maselli, M.A., Severi, C., 2015. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol 28, 203-209.

Chen, X., Herbert, J., 1995. Regional changes in c-fos expression in the basal forebrain and brainstem during adaptation to repeated stress: correlations with cardiovascular, hypothermic and endocrine responses. Neuroscience 64, 675-685.

Christianson, J.P., Paul, E.D., Irani, M., Thompson, B.M., Kubala, K.H., Yirmiya, R., Watkins, L.R., Maier, S.F., 2008. The role of prior stressor controllability and the dorsal raphé nucleus in sucrose preference and social exploration. Behav Brain Res 193, 87-93.

Cizza, G., Primma, S., Coyle, M., Gourgiotis, L., Csako, G., 2010. Depression and osteoporosis: a research synthesis with meta-analysis. Horm Metab Res 42, 467-482.

Clapcote, S.J., Roder, J.C., 2005. Simplex PCR assay for sex determination in mice. Biotechniques 38, 702, 704, 706.

Clayborne, Z.M., Gilman, S.E., Khandaker, G.M., Colman, I., 2024. Associations between prenatal stress with offspring inflammation, depression and anxiety. Psychoneuroendocrinology 169, 107162.

Clayborne, Z.M., Zou, R., Gilman, S.E., Khandaker, G.M., Fell, D.B., Colman, I., El Marroun, H., 2023. Associations between prenatal maternal stress, maternal inflammation during pregnancy, and children's internalizing and externalizing symptoms throughout childhood. Brain Behav Immun 114, 165-172.

Clemente, J.C., Pehrsson, E.C., Blaser, M.J., Sandhu, K., Gao, Z., Wang, B., Magris, M., Hidalgo, G., Contreras, M., Noya-Alarcón, Ó., Lander, O., McDonald, J., Cox, M., Walter, J., Oh, P.L., Ruiz, J.F., Rodriguez, S., Shen, N., Song, S.J., Metcalf, J., Knight, R., Dantas, G., Dominguez-Bello, M.G., 2015. The microbiome of uncontacted Amerindians. Sci Adv 1.

Coker, A.L., Smith, P.H., Bethea, L., King, M.R., McKeown, R.E., 2000. Physical health consequences of physical and psychological intimate partner violence. Arch Fam Med 9, 451-457.

Collison, L.W., Vignali, D.A., 2011. In vitro Treg suppression assays. Methods Mol Biol 707, 21-37.

Covic, T., Cumming, S.R., Pallant, J.F., Manolios, N., Emery, P., Conaghan, P.G., Tennant, A., 2012. Depression and anxiety in patients with rheumatoid arthritis: prevalence rates based on a comparison of the Depression, Anxiety and Stress Scale (DASS) and the hospital, Anxiety and Depression Scale (HADS). BMC Psychiatry 12, 6.

Cryan, J.F., Holmes, A., 2005. The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4, 775-790.

Cushing, K., Alvarado, D.M., Ciorba, M.A., 2015. Butyrate and Mucosal Inflammation: New Scientific Evidence Supports Clinical Observation. Clin Transl Gastroenterol 6, e108.

Dalile, B., Van Oudenhove, L., Vervliet, B., Verbeke, K., 2019. The role of short-chain fatty acids in microbiota-gut-brain communication. Nat Rev Gastroenterol Hepatol 16, 461-478.

Danese, A., McEwen, B.S., 2012. Adverse childhood experiences, allostasis, allostatic load, and age-related disease. Physiol Behav 106, 29-39.

Dantzer, R., 2017. Role of the Kynurenine Metabolism Pathway in Inflammation-Induced Depression: Preclinical Approaches. Curr Top Behav Neurosci 31, 117-138.

Daskalakis, N.P., Bagot, R.C., Parker, K.J., Vinkers, C.H., de Kloet, E.R., 2013. The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. Psychoneuroendocrinology 38, 1858-1873.

Davis, E.P., Narayan, A.J., 2020. Pregnancy as a period of risk, adaptation, and resilience for mothers and infants. Dev Psychopathol 32, 1625-1639.

de Bruijn, A.T., van Bakel, H.J., van Baar, A.L., 2009. Sex differences in the relation between prenatal maternal emotional complaints and child outcome. Early Hum Dev 85, 319-324.

de Kloet, C.S., Vermetten, E., Geuze, E., Kavelaars, A., Heijnen, C.J., Westenberg, H.G., 2006. Assessment of HPA-axis function in posttraumatic stress disorder: pharmacological and non-pharmacological challenge tests, a review. J Psychiatr Res 40, 550-567.

de Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6, 463-475.

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A.J., Green, W., Denou, E., Silva, M.A., Santacruz, A., Sanz, Y., Surette, M.G., Verdu, E.F., Collins, S.M., Bercik, P., 2015. Microbiota and host determinants of behavioural phenotype in maternally separated mice. Nat Commun 6, 7735.

de Punder, K., Pruimboom, L., 2015. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. Front Immunol 6, 223.

Dealberto, M.J., 2010. Ethnic origin and increased risk for schizophrenia in immigrants to countries of recent and longstanding immigration. Acta Psychiatr Scand 121, 325-339.

Depner, M., Taft, D.H., Kirjavainen, P.V., Kalanetra, K.M., Karvonen, A.M., Peschel, S., Schmausser-Hechfellner, E., Roduit, C., Frei, R., Lauener, R., Divaret-Chauveau, A., Dalphin, J.C., Riedler, J., Roponen, M., Kabesch, M., Renz, H., Pekkanen, J., Farquharson, F.M., Louis, P., Mills, D.A., von Mutius, E., Ege, M.J., 2020. Maturation of the gut microbiome during the first year of life contributes to the protective farm effect on childhood asthma. Nat Med 26, 1766-1775.

Desmond, L.W., Dawud, L.M., Kessler, L.R., Akonom, T., Hunter, E.A.H., Holbrook, E.M., Andersen, N.D., Sterrett, J.D., Boateng, D.A., Stuart, B.J., Guerrero, L., Gebert, M.J., Tsai, P.S., Langgartner, D., Reber, S.O., Frank, M.G., Lowry, C.A., 2024. Protective effects of Mycobacterium vaccae ATCC 15483 against "Western"-style diet-induced weight gain and visceral adiposity in adolescent male mice. Brain Behav Immun 125, 249-267.

Desmond, L.W., Holbrook, E.M., Wright, C.T.O., Zambrano, C.A., Stamper, C.E., Bohr, A.D., Frank, M.G., Podell, B.K., Moreno, J.A., MacDonald, A.S., Reber, S.O., Hernández-Pando, R., Lowry, C.A., 2023. Effects of Mycobacterium vaccae NCTC 11659 and Lipopolysaccharide Challenge on Polarization of Murine BV-2 Microglial Cells. Int J Mol Sci 25.

Dhaliwal, S.K., Dabelea, D., Lee-Winn, A.E., Crume, T., Wilkening, G., Perng, W., 2023. Maternal psychosocial stress during pregnancy and offspring neurobehavioral outcomes during early childhood in the Healthy Start Study. Ann Epidemiol 86, 16-24.e13.

Dimsdale, J.E., 2008. Psychological stress and cardiovascular disease. J Am Coll Cardiol 51, 1237-1246.

Dinan, T.G., Cryan, J.F., 2017. Brain-Gut-Microbiota Axis and Mental Health. Psychosom Med 79, 920-926.

Diz-Chaves, Y., Astiz, M., Bellini, M.J., Garcia-Segura, L.M., 2013. Prenatal stress increases the expression of proinflammatory cytokines and exacerbates the inflammatory response to LPS in the hippocampal formation of adult male mice. Brain Behav Immun 28, 196-206.

Duivis, H.E., de Jonge, P., Penninx, B.W., Na, B.Y., Cohen, B.E., Whooley, M.A., 2011. Depressive symptoms, health behaviors, and subsequent inflammation in patients with coronary heart disease: prospective findings from the heart and soul study. Am J Psychiatry 168, 913-920.

Dulberger, C.L., Rubin, E.J., Boutte, C.C., 2020. The mycobacterial cell envelope - a moving target. Nat Rev Microbiol 18, 47-59.

Eaton, N.R., Keyes, K.M., Krueger, R.F., Balsis, S., Skodol, A.E., Markon, K.E., Grant, B.F., Hasin, D.S., 2012. An invariant dimensional liability model of gender differences in mental disorder prevalence: evidence from a national sample. J Abnorm Psychol 121, 282-288.

Eeckhaut, V., Machiels, K., Perrier, C., Romero, C., Maes, S., Flahou, B., Steppe, M., Haesebrouck, F., Sas, B., Ducatelle, R., Vermeire, S., Van Immerseel, F., 2013. Butyricicoccus pullicaecorum in inflammatory bowel disease. Gut 62, 1745-1752.

Ege, M.J., Herzum, I., Büchele, G., Krauss-Etschmann, S., Lauener, R.P., Roponen, M., Hyvärinen, A., Vuitton, D.A., Riedler, J., Brunekreef, B., Dalphin, J.-C., Braun-Fahrländer, C., Pekkanen, J., Renz, H., von Mutius, E., 2008. Prenatal exposure to a

farm environment modifies atopic sensitization at birth. Journal of Allergy and Clinical Immunology 122, 407-412.e404.

Eldeirawi, K., McConnell, R., Furner, S., Freels, S., Stayner, L., Hernandez, E., Amoruso, L., Torres, S., Persky, V.W., 2009. Associations of doctor-diagnosed asthma with immigration status, age at immigration, and length of residence in the United States in a sample of Mexican American School Children in Chicago. J Asthma 46, 796-802.

Ellul, P., Mariotti-Ferrandiz, E., Leboyer, M., Klatzmann, D., 2018. Regulatory T Cells As Supporters of Psychoimmune Resilience: Toward Immunotherapy of Major Depressive Disorder. Front Neurol 9, 167.

Faden, H., 2022. The Role of Faecalibacterium, Roseburia, and Butyrate in Inflammatory Bowel Disease. Dig Dis 40, 793-795.

Fasano, A., Chassaing, B., Haller, D., Flores Ventura, E., Carmen-Collado, M., Pastor, N., Koren, O., Berni Canani, R., 2024. Microbiota during pregnancy and early life: role in maternal-neonatal outcomes based on human evidence. Gut Microbes 16, 2392009.

Figliuolo, V.R., Dos Santos, L.M., Abalo, A., Nanini, H., Santos, A., Brittes, N.M., Bernardazzi, C., de Souza, H.S.P., Vieira, L.Q., Coutinho-Silva, R., Coutinho, C., 2017. Sulfate-reducing bacteria stimulate gut immune responses and contribute to inflammation in experimental colitis. Life Sci 189, 29-38.

Finckh, A., Gilbert, B., Hodkinson, B., Bae, S.C., Thomas, R., Deane, K.D., Alpizar-Rodriguez, D., Lauper, K., 2022. Global epidemiology of rheumatoid arthritis. Nat Rev Rheumatol 18, 591-602.

Finnell, J.E., Lombard, C.M., Padi, A.R., Moffitt, C.M., Wilson, L.B., Wood, C.S., Wood, S.K., 2017. Physical versus psychological social stress in male rats reveals distinct cardiovascular, inflammatory and behavioral consequences. PLoS One 12, e0172868.

Flannery, R.B., 2022. Psychological Trauma and the Trauma Surgeon. Psychiatr Q 93, 27-33.

Foertsch, S., Fuchsl, A.M., Faller, S.D., Holzer, H., Langgartner, D., Messmann, J., Strauss, G., Reber, S.O., 2017a. Splenic glucocorticoid resistance following psychosocial stress requires physical injury. Sci Rep 7, 15730.

Foertsch, S., Haffner-Luntzer, M., Kroner, J., Gross, F., Kaiser, K., Erber, M., Reber, S.O., Ignatius, A., 2017b. Chronic psychosocial stress disturbs long-bone growth in adolescent mice. Dis Model Mech 10, 1399-1409.

Foertsch, S., Lackner, I., Weber, B., Fuchsl, A.M., Langgartner, D., Wirkert, E., Peters, S., Fois, G., Pressmar, J., Fegert, J.M., Frick, M., Gundel, H., Kalbitz, M., Reber, S.O., 2019. Sensory contact to the stressor prevents recovery from structural and functional heart damage following psychosocial trauma. Brain Behav Immun 80, 667-677.

Foertsch, S., Langgartner, D., Reber, S.O., 2020. Abdominal surgery prior to chronic psychosocial stress promotes spleen cell (re)activity and glucocorticoid resistance. Sci Rep 10, 6917.

Foertsch, S., Reber, S.O., 2020. The role of physical trauma in social stress-induced immune activation. Neurosci Biobehav Rev 113, 169-178.

Fonken, L.K., Frank, M.G., D'Angelo, H.M., Heinze, J.D., Watkins, L.R., Lowry, C.A., Maier, S.F., 2018a. Mycobacterium vaccae immunization protects aged rats from surgery-elicited neuroinflammation and cognitive dysfunction. Neurobiol Aging 71, 105-114.

Fonken, L.K., Frank, M.G., Gaudet, A.D., Maier, S.F., 2018b. Stress and aging act through common mechanisms to elicit neuroinflammatory priming. Brain, Behavior, and Immunity 73, 133-148.

Fontenot, J.D., Gavin, M.A., Rudensky, A.Y., 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4, 330-336.

Förger, F., Villiger, P.M., 2020. Immunological adaptations in pregnancy that modulate rheumatoid arthritis disease activity. Nat Rev Rheumatol 16, 113-122.

Forsythe, P., Kunze, W., Bienenstock, J., 2016. Moody microbes or fecal phrenology: what do we know about the microbiota-gut-brain axis? BMC Med 14, 58.

Fowler, D.W., Copier, J., Wilson, N., Dalgleish, A.G., Bodman-Smith, M.D., 2012. Mycobacteria activate γδ T-cell anti-tumour responses via cytokines from type 1 myeloid dendritic cells: a mechanism of action for cancer immunotherapy. Cancer Immunol Immunother 61, 535-547.

Foxx, C.L., Heinze, J.D., González, A., Vargas, F., Baratta, M.V., Elsayed, A.I., Stewart, J.R., Loupy, K.M., Arnold, M.R., Flux, M.C., Sago, S.A., Siebler, P.H., Milton, L.N., Lieb, M.W., Hassell, J.E., Smith, D.G., Lee, K.A.K., Appiah, S.A., Schaefer, E.J., Panitchpakdi, M., Sikora, N.C., Weldon, K.C., Stamper, C.E., Schmidt, D., Duggan, D.A., Mengesha, Y.M., Ogbaselassie, M., Nguyen, K.T., Gates, C.A., Schnabel, K., Tran, L., Jones, J.D., Vitaterna, M.H., Turek, F.W., Fleshner, M., Dorrestein, P.C., Knight, R., Wright, K.P., Lowry, C.A., 2020. Effects of Immunization With the Soil-Derived Bacterium Mycobacterium vaccae on Stress Coping Behaviors and Cognitive Performance in a "Two Hit" Stressor Model. Front Physiol 11, 524833.

Frank, M.G., Fonken, L.K., Dolzani, S.D., Annis, J.L., Siebler, P.H., Schmidt, D., Watkins, L.R., Maier, S.F., Lowry, C.A., 2018a. Immunization with Mycobacterium vaccae induces an anti-inflammatory milieu in the CNS: Attenuation of stress-induced microglial priming, alarmins and anxiety-like behavior. Brain Behav Immun 73, 352-363.

Frank, M.G., Fonken, L.K., Watkins, L.R., Maier, S.F., Lowry, C.A., 2018b. Could Probiotics Be Used to Mitigate Neuroinflammation? ACS Chemical Neuroscience.

Füchsl, A.M., Langgartner, D., Reber, S.O., 2013. Mechanisms underlying the increased plasma ACTH levels in chronic psychosocially stressed male mice. PLoS One 8, e84161.

Fuhler, G.M., 2020. The immune system and microbiome in pregnancy. Best Pract Res Clin Gastroenterol 44-45, 101671.

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., Takahashi, M., Fukuda, N.N., Murakami, S., Miyauchi, E., Hino, S., Atarashi, K., Onawa, S., Fujimura, Y., Lockett, T., Clarke, J.M., Topping, D.L., Tomita, M., Hori, S., Ohara, O., Morita, T., Koseki, H., Kikuchi, J., Honda, K., Hase, K., Ohno, H., 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 504, 446-450.

Galley, J.D., Mashburn-Warren, L., Blalock, L.C., Lauber, C.L., Carroll, J.E., Ross, K.M., Hobel, C., Coussons-Read, M., Dunkel Schetter, C., Gur, T.L., 2023. Maternal anxiety, depression and stress affects offspring gut microbiome diversity and bifidobacterial abundances. Brain Behav Immun 107, 253-264.

Gao, F., Guo, R., Ma, Q., Li, Y., Wang, W., Fan, Y., Ju, Y., Zhao, B., Gao, Y., Qian, L., Yang, Z., He, X., Jin, X., Liu, Y., Peng, Y., Chen, C., Chen, Y., Gao, C., Zhu, F., Ma, X., 2022. Stressful events induce long-term gut microbiota dysbiosis and associated post-traumatic stress symptoms in healthcare workers fighting against COVID-19. J Affect Disord 303, 187-195.

García-Ródenas, C.L., Bergonzelli, G.E., Nutten, S., Schumann, A., Cherbut, C., Turini, M., Ornstein, K., Rochat, F., Corthésy-Theulaz, I., 2006. Nutritional approach to restore impaired intestinal barrier function and growth after neonatal stress in rats. J Pediatr Gastroenterol Nutr 43, 16-24.

Gautam, A., Kumar, R., Chakraborty, N., Muhie, S., Hoke, A., Hammamieh, R., Jett, M., 2018. Altered fecal microbiota composition in all male aggressor-exposed rodent model simulating features of post-traumatic stress disorder. J Neurosci Res 96, 1311-1323.

Gebara, M.A., Shea, M.L., Lipsey, K.L., Teitelbaum, S.L., Civitelli, R., Müller, D.J., Reynolds, C.F., 3rd, Mulsant, B.H., Lenze, E.J., 2014. Depression, antidepressants, and bone health in older adults: a systematic review. J Am Geriatr Soc 62, 1434-1441.

Gebauer, D., Schimmele, T., Mazzari, G., Krüger, B.T., Zemui, M., Ignatius, A., Langgartner, D., Haffner-Luntzer, M., Reber, S.O., 2025. The sex-specific effects of early life adversity and chronic psychosocial stress during adulthood on bone are mitigated by Mycobacterium vaccae NCTC 11659 in mice. Neuroimmunomodulation, 1-25.

Gergianaki, I., Bortoluzzi, A., Bertsias, G., 2018. Update on the epidemiology, risk factors, and disease outcomes of systemic lupus erythematosus. Best Pract Res Clin Rheumatol 32, 188-205.

Gitau, R., Cameron, A., Fisk, N.M., Glover, V., 1998. Fetal exposure to maternal cortisol. Lancet 352, 707-708.

Glaesmer, H., Brähler, E., Gündel, H., Riedel-Heller, S.G., 2011. The association of traumatic experiences and posttraumatic stress disorder with physical morbidity in old age: a German population-based study. Psychosom Med 73, 401-406.

Glaesmer, H., Kaiser, M., Bräehler, E., Freyberger, H.J., Kuwert, P., 2012. Posttraumatic stress disorder and its comorbidity with depression and somatisation in the elderly - a German community-based study. Aging Ment Health 16, 403-412.

Gola, H., Engler, H., Sommershof, A., Adenauer, H., Kolassa, S., Schedlowski, M., Groettrup, M., Elbert, T., Kolassa, I.T., 2013. Posttraumatic stress disorder is associated with an enhanced spontaneous production of pro-inflammatory cytokines by peripheral blood mononuclear cells. BMC Psychiatry 13, 40.

Goldstein, J.M., Cohen, J.E., Mareckova, K., Holsen, L., Whitfield-Gabrieli, S., Gilman, S.E., Buka, S.L., Hornig, M., 2021. Impact of prenatal maternal cytokine exposure on sex differences in brain circuitry regulating stress in offspring 45 years later. Proc Natl Acad Sci U S A 118.

Gophna, U., Konikoff, T., Nielsen, H.B., 2017. Oscillospira and related bacteria - From metagenomic species to metabolic features. Environ Microbiol 19, 835-841.

Grainger, J.R., Smith, K.A., Hewitson, J.P., McSorley, H.J., Harcus, Y., Filbey, K.J., Finney, C.A., Greenwood, E.J., Knox, D.P., Wilson, M.S., Belkaid, Y., Rudensky, A.Y., Maizels, R.M., 2010. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-β pathway. J Exp Med 207, 2331-2341.

Grenham, S., Clarke, G., Cryan, J.F., Dinan, T.G., 2011. Brain-gut-microbe communication in health and disease. Front Physiol 2, 94.

Gros, D.F., Allan, N.P., Koscinski, B., Keller, S., Acierno, R., 2023. Influence of comorbid social anxiety disorder in PTSD treatment outcomes for Prolonged Exposure in female military sexual trauma survivors with PTSD. J Clin Psychol 79, 1039-1050.

Grubbs, F.E., 1969. Procedures for Detecting Outlying Observations in Samples. Technometrics 11, 1-21.

Gryaznova, M., Dvoretskaya, Y., Burakova, I., Syromyatnikov, M., Popov, E., Kokina, A., Mikhaylov, E., Popov, V., 2022. Dynamics of Changes in the Gut Microbiota of Healthy Mice Fed with Lactic Acid Bacteria and Bifidobacteria. Microorganisms 10.

Gu, S., Chen, D., Zhang, J.N., Lv, X., Wang, K., Duan, L.P., Nie, Y., Wu, X.L., 2013. Bacterial community mapping of the mouse gastrointestinal tract. PLoS One 8, e74957.

Gur, T.L., Palkar, A.V., Rajasekera, T., Allen, J., Niraula, A., Godbout, J., Bailey, M.T., 2019. Prenatal stress disrupts social behavior, cortical neurobiology and commensal microbes in adult male offspring. Behav Brain Res 359, 886-894.

Gur, T.L., Shay, L., Palkar, A.V., Fisher, S., Varaljay, V.A., Dowd, S., Bailey, M.T., 2017. Prenatal stress affects placental cytokines and neurotrophins, commensal microbes, and anxiety-like behavior in adult female offspring. Brain Behav Immun 64, 50-58.

Habib, N., Pasha, M.A., Tang, D.D., 2022. Current Understanding of Asthma Pathogenesis and Biomarkers. Cells 11.

Hadizadeh, M., Hamidi, G.A., Salami, M., 2019. Probiotic supplementation improves the cognitive function and the anxiety-like behaviors in the stressed rats. Iran J Basic Med Sci 22, 506-514.

Haffner-Luntzer, M., Foertsch, S., Fischer, V., Prystaz, K., Tschaffon, M., Mödinger, Y., Bahney, C.S., Marcucio, R.S., Miclau, T., Ignatius, A., Reber, S.O., 2019. Chronic psychosocial stress compromises the immune response and endochondral ossification during bone fracture healing via β-AR signaling. Proc Natl Acad Sci U S A 116, 8615-8622.

Haller, J., Fuchs, E., Halász, J., Makara, G.B., 1999. Defeat is a major stressor in males while social instability is stressful mainly in females: towards the development of a social stress model in female rats. Brain Res Bull 50, 33-39.

Hamer, H.M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F.J., Brummer, R.J., 2008. Review article: the role of butyrate on colonic function. Aliment Pharmacol Ther 27, 104-119.

Hanski, I., von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., Karisola, P., Auvinen, P., Paulin, L., Mäkelä, M.J., Vartiainen, E., Kosunen, T.U., Alenius, H., Haahtela, T., 2012. Environmental biodiversity, human microbiota, and allergy are interrelated. Proc Natl Acad Sci U S A 109, 8334-8339.

Hassell, J.E., Jr., Fox, J.H., Arnold, M.R., Siebler, P.H., Lieb, M.W., Schmidt, D., Spratt, E.J., Smith, T.M., Nguyen, K.T., Gates, C.A., Holmes, K.S., Schnabel, K.S., Loupy, K.M., Erber, M., Lowry, C.A., 2019. Treatment with a heat-killed preparation of Mycobacterium vaccae after fear conditioning enhances fear extinction in the fear-potentiated startle paradigm. Brain Behav Immun 81, 151-160.

Hebert, J.C., Radford-Smith, D.E., Probert, F., Ilott, N., Chan, K.W., Anthony, D.C., Burnet, P.W.J., 2021. Mom's diet matters: Maternal prebiotic intake in mice reduces anxiety and alters brain gene expression and the fecal microbiome in offspring. Brain Behav Immun 91, 230-244.

Heim, C., Nater, U.M., Maloney, E., Boneva, R., Jones, J.F., Reeves, W.C., 2009. Childhood trauma and risk for chronic fatigue syndrome: association with neuroendocrine dysfunction. Arch Gen Psychiatry 66, 72-80.

Heinrichs, S.C., Koob, G.F., 2006. Application of experimental stressors in laboratory rodents. Curr Protoc Neurosci Chapter 8, Unit8.4.

Hemmings, S.M.J., Malan-Müller, S., van den Heuvel, L.L., Demmitt, B.A., Stanislawski, M.A., Smith, D.G., Bohr, A.D., Stamper, C.E., Hyde, E.R., Morton, J.T., Marotz, C.A.,

Siebler, P.H., Braspenning, M., Van Criekinge, W., Hoisington, A.J., Brenner, L.A., Postolache, T.T., McQueen, M.B., Krauter, K.S., Knight, R., Seedat, S., Lowry, C.A., 2017. The Microbiome in Posttraumatic Stress Disorder and Trauma-Exposed Controls: An Exploratory Study. Psychosom Med 79, 936-946.

Hillerer, K.M., Neumann, I.D., Slattery, D.A., 2012. From stress to postpartum mood and anxiety disorders: how chronic peripartum stress can impair maternal adaptations. Neuroendocrinology 95, 22-38.

Hillerer, K.M., Reber, S.O., Neumann, I.D., Slattery, D.A., 2011. Exposure to chronic pregnancy stress reverses peripartum-associated adaptations: implications for postpartum anxiety and mood disorders. Endocrinology 152, 3930-3940.

Hodes, G.E., Epperson, C.N., 2019. Sex Differences in Vulnerability and Resilience to Stress Across the Life Span. Biol Psychiatry 86, 421-432.

Hodes, G.E., Pfau, M.L., Leboeuf, M., Golden, S.A., Christoffel, D.J., Bregman, D., Rebusi, N., Heshmati, M., Aleyasin, H., Warren, B.L., Lebonté, B., Horn, S., Lapidus, K.A., Stelzhammer, V., Wong, E.H., Bahn, S., Krishnan, V., Bolaños-Guzman, C.A., Murrough, J.W., Merad, M., Russo, S.J., 2014. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc Natl Acad Sci U S A 111, 16136-16141.

Hoffmann, C., Dollive, S., Grunberg, S., Chen, J., Li, H., Wu, G.D., Lewis, J.D., Bushman, F.D., 2013. Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. PLoS One 8, e66019.

Holmes, M.C., Abrahamsen, C.T., French, K.L., Paterson, J.M., Mullins, J.J., Seckl, J.R., 2006. The mother or the fetus? 11beta-hydroxysteroid dehydrogenase type 2 null mice provide evidence for direct fetal programming of behavior by endogenous glucocorticoids. J Neurosci 26, 3840-3844.

Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. Science 299, 1057-1061.

Hou, J.K., El-Serag, H., Thirumurthi, S., 2009. Distribution and manifestations of inflammatory bowel disease in Asians, Hispanics, and African Americans: a systematic review. Am J Gastroenterol 104, 2100-2109.

Howerton, C.L., Bale, T.L., 2012. Prenatal programing: at the intersection of maternal stress and immune activation. Horm Behav 62, 237-242.

Howerton, C.L., Morgan, C.P., Fischer, D.B., Bale, T.L., 2013. O-GlcNAc transferase (OGT) as a placental biomarker of maternal stress and reprogramming of CNS gene transcription in development. Proc Natl Acad Sci U S A 110, 5169-5174.

Howren, M.B., Lamkin, D.M., Suls, J., 2009. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med 71, 171-186.

Hu, X., Li, Y., Wu, J., Zhang, H., Huang, Y., Tan, X., Wen, L., Zhou, X., Xie, P., Olasunkanmi, O.I., Zhou, J., Sun, Z., Liu, M., Zhang, G., Yang, J., Zheng, P., Xie, P., 2023. Changes of gut microbiota reflect the severity of major depressive disorder: a cross sectional study. Transl Psychiatry 13, 137.

Hussen, H.I., Persson, M., Moradi, T., 2013a. The trends and the risk of type 1 diabetes over the past 40 years: an analysis by birth cohorts and by parental migration background in Sweden. BMJ Open 3, e003418.

Hussen, H.I., Yang, D., Cnattingius, S., Moradi, T., 2013b. Type I diabetes among children and young adults: the role of country of birth, socioeconomic position and sex. Pediatr Diabetes 14, 138-148.

Ishikawa, E., Mori, D., Yamasaki, S., 2017. Recognition of Mycobacterial Lipids by Immune Receptors. Trends Immunol 38, 66-76.

Iwata, M., Ota, K.T., Duman, R.S., 2013. The inflammasome: pathways linking psychological stress, depression, and systemic illnesses. Brain Behav Immun 31, 105-114.

Jackson, S.S., Marks, M.A., Katki, H.A., Cook, M.B., Hyun, N., Freedman, N.D., Kahle, L.L., Castle, P.E., Graubard, B.I., Chaturvedi, A.K., 2022. Sex disparities in the incidence of 21 cancer types: Quantification of the contribution of risk factors. Cancer 128, 3531-3540.

Jarcho, M.R., Massner, K.J., Eggert, A.R., Wichelt, E.L., 2016. Behavioral and physiological response to onset and termination of social instability in female mice. Horm Behav 78, 135-140.

Jašarević, E., Bale, T.L., 2019. Prenatal and postnatal contributions of the maternal microbiome on offspring programming. Front Neuroendocrinol 55, 100797.

Jašarević, E., Howard, C.D., Misic, A.M., Beiting, D.P., Bale, T.L., 2017. Stress during pregnancy alters temporal and spatial dynamics of the maternal and offspring microbiome in a sex-specific manner. Sci Rep 7, 44182.

Johnson, J.L., Kamya, R.M., Okwera, A., Loughlin, A.M., Nyole, S., Hom, D.L., Wallis, R.S., Hirsch, C.S., Wolski, K., Foulds, J., Mugerwa, R.D., Ellner, J.J., 2000. Randomized controlled trial of Mycobacterium vaccae immunotherapy in non-human immunodeficiency virus-infected ugandan adults with newly diagnosed pulmonary tuberculosis. The Uganda-Case Western Reserve University Research Collaboration. J Infect Dis 181, 1304-1312.

Jones, K.D., Berkley, J.A., Warner, J.O., 2010. Perinatal nutrition and immunity to infection. Pediatr Allergy Immunol 21, 564-576.

José Neto, N., Duarte Brito, M., Gomes, C.D.S., Corrêa, L., Guerra, G.C.B., Guerra, R.O., 2025. Gut microbiota dysbiosis, sarcopenia, osteoporosis and osteosarcopenia in older people: A systematic review protocol. PLoS One 20, e0313193.

Kajantie, E., Phillips, D.I., 2006. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. Psychoneuroendocrinology 31, 151-178.

Kalinichenko, L.S., Smaga, I., Filip, M., Lenz, B., Kornhuber, J., Müller, C.P., 2023. Sex-specific effects of different types of prenatal stress on foetal testosterone levels and NMDA expression in mice. Behav Brain Res 439, 114225.

Kappel, S., Hawkins, P., Mendl, M.T., 2017. To Group or Not to Group? Good Practice for Housing Male Laboratory Mice. Animals (Basel) 7.

Kashfi, S.S., Abdollahi, G., Hassanzadeh, J., Mokarami, H., Khani Jeihooni, A., 2022. The relationship between osteoporosis and depression. Sci Rep 12, 11177.

Keen, D.V., Reid, F.D., Arnone, D., 2010. Autism, ethnicity and maternal immigration. Br J Psychiatry 196, 274-281.

Kelly, J.R., Borre, Y., C, O.B., Patterson, E., El Aidy, S., Deane, J., Kennedy, P.J., Beers, S., Scott, K., Moloney, G., Hoban, A.E., Scott, L., Fitzgerald, P., Ross, P., Stanton, C., Clarke, G., Cryan, J.F., Dinan, T.G., 2016. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. J Psychiatr Res 82, 109-118.

Kempter, E., Amoroso, M., Duffner, H.L., Werner, A.M., Langgartner, D., Kupfer, S., Reber, S.O., 2021. Changes in Functional Glucocorticoid Sensitivity of Isolated Splenocytes Induced by Chronic Psychosocial Stress - A Time Course Study. Front Immunol 12, 753822.

Kempter, E., Amoroso, M., Kupfer, S., Lupu, L., Kustermann, M., Scheurer, J., Baumann, B., Wirth, T., Gündel, H., Straub, R.H., Strauß, G., Huber-Lang, M., Langgartner, D., Reber, S.O., 2023. The PMN-MDSC - A key player in glucocorticoid resistance following combined physical and psychosocial trauma. Brain Behav Immun 108, 148-161.

Kessler, R.C., 2003. Epidemiology of women and depression. J Affect Disord 74, 5-13.

Kessler, R.C., McGonagle, K.A., Swartz, M., Blazer, D.G., Nelson, C.B., 1993. Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence. J Affect Disord 29, 85-96.

Khan, M.F., Khodve, G., Yadav, S., Mallick, K., Banerjee, S., 2025. Probiotic treatment improves post-traumatic stress disorder outcomes in mice. Behav Brain Res 476, 115246.

Khandaker, G.M., Pearson, R.M., Zammit, S., Lewis, G., Jones, P.B., 2014. Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study. JAMA Psychiatry 71, 1121-1128.

Kiecolt-Glaser, J.K., Wilson, S.J., Bailey, M.L., Andridge, R., Peng, J., Jaremka, L.M., Fagundes, C.P., Malarkey, W.B., Laskowski, B., Belury, M.A., 2018. Marital distress,

depression, and a leaky gut: Translocation of bacterial endotoxin as a pathway to inflammation. Psychoneuroendocrinology 98, 52-60.

Kimmel, M.C., Verosky, B., Chen, H.J., Davis, O., Gur, T.L., 2024. The Maternal Microbiome as a Map to Understanding the Impact of Prenatal Stress on Offspring Psychiatric Health. Biol Psychiatry 95, 300-309.

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom Med 61, 154-162.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76-81.

Kivimaki, M., Shipley, M.J., Batty, G.D., Hamer, M., Akbaraly, T.N., Kumari, M., Jokela, M., Virtanen, M., Lowe, G.D., Ebmeier, K.P., Brunner, E.J., Singh-Manoux, A., 2014. Long-term inflammation increases risk of common mental disorder: a cohort study. Mol Psychiatry 19, 149-150.

Klengel, T., Dias, B.G., Ressler, K.J., 2016. Models of Intergenerational and Transgenerational Transmission of Risk for Psychopathology in Mice. Neuropsychopharmacology 41, 219-231.

Koolhaas, J.M., Bartolomucci, A., Buwalda, B., de Boer, S.F., Flugge, G., Korte, S.M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Wohr, M., Fuchs, E., 2011. Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev 35, 1291-1301.

Koren, O., Konnikova, L., Brodin, P., Mysorekar, I.U., Collado, M.C., 2024. The maternal gut microbiome in pregnancy: implications for the developing immune system. Nat Rev Gastroenterol Hepatol 21, 35-45.

Kuehner, C., 2017. Why is depression more common among women than among men? Lancet Psychiatry 4, 146-158.

Lafuse, W.P., Gearinger, R., Fisher, S., Nealer, C., Mackos, A.R., Bailey, M.T., 2017. Exposure to a Social Stressor Induces Translocation of Commensal Lactobacilli to the Spleen and Priming of the Innate Immune System. J Immunol 198, 2383-2393.

Langgartner, D., Amoroso, M., Kempter, E., Kustermann, M., Scheurer, J., Lowry, C.A., Strauß, G., Reber, S.O., 2023a. Mycobacterium vaccae protects against glucocorticoid resistance resulting from combined physical and psychosocial trauma in mice. Brain Behav Immun 109, 221-234.

Langgartner, D., Füchsl, A.M., Kaiser, L.M., Meier, T., Foertsch, S., Buske, C., Reber, S.O., Mulaw, M.A., 2018a. Biomarkers for classification and class prediction of stress in a murine model of chronic subordination stress. PLoS One 13, e0202471.

Langgartner, D., Fuchsl, A.M., Uschold-Schmidt, N., Slattery, D.A., Reber, S.O., 2015. Chronic subordinate colony housing paradigm: a mouse model to characterize the consequences of insufficient glucocorticoid signaling. Front Psychiatry 6, 18.

Langgartner, D., Koenen, M., Kupfer, S., Glogger, L., Kurz, L., Perez-Rivas, L.G., Theodoropoulou, M., Noll-Hussong, M., Vettorazzi, S., Tuckermann, J., Reber, S.O., 2023b. Intact GR dimerization is critical for restraining plasma ACTH levels during chronic psychosocial stress. Neurobiol Stress 24, 100541.

Langgartner, D., Lowry, C.A., Reber, S.O., 2019. Old Friends, immunoregulation, and stress resilience. Pflugers Arch 471, 237-269.

Langgartner, D., Palmer, A., Rittlinger, A., Reber, S.O., Huber-Lang, M., 2018b. Effects of Prior Psychosocial Trauma on Subsequent Immune Response After Experimental Thorax Trauma. Shock 49, 690-697.

Langgartner, D., Peterlik, D., Foertsch, S., Füchsl, A.M., Brokmann, P., Flor, P.J., Shen, Z., Fox, J.G., Uschold-Schmidt, N., Lowry, C.A., Reber, S.O., 2017. Individual differences in stress vulnerability: The role of gut pathobionts in stress-induced colitis. Brain Behav Immun 64, 23-32.

Langgartner, D., Weimer, K., Brunner-Weisser, J., Winkler, R., Mannes, M., Huber-Lang, M., Sterrett, J.D., Lowry, C.A., Rohleder, N., Bajrami, B., Luippold, A.H., Groß, A., Kestler, H.A., Tost, H., Meyer-Lindenberg, A., Gündel, H., Jarczok, M.N., Reber, S.O., 2025. Pawsitive impact: How pet contact ameliorates adult inflammatory stress responses in individuals raised in an urban environment. Brain Behav Immun 127, 217-228.

Langgartner, D., Weiss, A.J., Amoroso, M., Sterrett, J.D., Lowry, C.A., Reber, S.O., 2024. Effects of repeated intragastric administrations with heat-inactivated Mycobacterium aurum DSM 33539 on the stress-induced aggravation of dextran sulfate sodium (DSS) colitis in C57BL/6N mice. Front Neurosci 18, 1488603.

Langgartner, D., Zambrano, C.A., Heinze, J.D., Stamper, C.E., Böbel, T.S., Hackl, S.B., Jarczok, M.N., Rohleder, N., Rook, G.A., Gündel, H., Waller, C., Lowry, C.A., Reber, S.O., 2020. Association of the Salivary Microbiome With Animal Contact During Early Life and Stress-Induced Immune Activation in Healthy Participants. Front Psychiatry 11, 353.

Le Bert, N., Chain, B.M., Rook, G., Noursadeghi, M., 2011. DC priming by M. vaccae inhibits Th2 responses in contrast to specific TLR2 priming and is associated with selective activation of the CREB pathway. PLoS One 6, e18346.

Lee, J., d'Aigle, J., Atadja, L., Quaicoe, V., Honarpisheh, P., Ganesh, B.P., Hassan, A., Graf, J., Petrosino, J., Putluri, N., Zhu, L., Durgan, D.J., Bryan, R.M., Jr., McCullough, L.D., Venna, V.R., 2020. Gut Microbiota-Derived Short-Chain Fatty Acids Promote Poststroke Recovery in Aged Mice. Circ Res 127, 453-465.

Lehrer, A., Bressanelli, A., Wachsmann, V., Bottasso, O., Bay, M.L., Singh, M., Stanford, C., Stanford, J., 1998. Immunotherapy with Mycobacterium vaccae in the treatment of psoriasis. FEMS Immunol Med Microbiol 21, 71-77.

Leigh, S.J., Uhlig, F., Wilmes, L., Sanchez-Diaz, P., Gheorghe, C.E., Goodson, M.S., Kelley-Loughnane, N., Hyland, N.P., Cryan, J.F., Clarke, G., 2023. The impact of acute and chronic stress on gastrointestinal physiology and function: a microbiota-gut-brain axis perspective. J Physiol 601, 4491-4538.

Leung, J., Hang, L., Blum, A., Setiawan, T., Stoyanoff, K., Weinstock, J., 2012. Heligmosomoides polygyrus abrogates antigen-specific gut injury in a murine model of inflammatory bowel disease. Inflamm Bowel Dis 18, 1447-1455.

Li, H., Li, X., Jia, N., Cai, Q., Bai, Z., Chen, R., Song, T., Zhu, Z., Liu, J., 2008. NF-kappaB regulates prenatal stress-induced cognitive impairment in offspring rats. Behav Neurosci 122, 331-339.

Li, X., Sundquist, J., Hemminki, K., Sundquist, K., 2011. Risk of inflammatory bowel disease in first- and second-generation immigrants in Sweden: a nationwide follow-up study. Inflamm Bowel Dis 17, 1784-1791.

Li, Y., Horst, K., Greven, J., Mert, Ü., Lupu, L., Palmer, A., Doerfer, L., Zhao, Q., Zhang, X., Halbgebauer, R., Ignatius, A., Marzi, I., van Griensven, M., Balmayor, E., Hildebrand, F., Mollnes, T.E., Huber-Lang, M., 2024. Modulation of the hepatic RANK-RANKL-OPG axis by combined C5 and CD14 inhibition in a long-term polytrauma model. Front Immunol 15, 1434274.

Li, Y., Xiao, B., Qiu, W., Yang, L., Hu, B., Tian, X., Yang, H., 2010. Altered expression of CD4(+)CD25(+) regulatory T cells and its 5-HT(1a) receptor in patients with major depression disorder. J Affect Disord 124, 68-75.

Lindqvist, D., Wolkowitz, O.M., Mellon, S., Yehuda, R., Flory, J.D., Henn-Haase, C., Bierer, L.M., Abu-Amara, D., Coy, M., Neylan, T.C., Makotkine, I., Reus, V.I., Yan, X., Taylor, N.M., Marmar, C.R., Dhabhar, F.S., 2014. Proinflammatory milieu in combat-related PTSD is independent of depression and early life stress. Brain Behav Immun 42, 81-88.

Liu, L., Tian, F., Li, G.Y., Xu, W., Xia, R., 2022a. The effects and significance of gut microbiota and its metabolites on the regulation of osteoarthritis: Close coordination of gut-bone axis. Front Nutr 9, 1012087.

Liu, Y., Tian, S., Ning, B., Huang, T., Li, Y., Wei, Y., 2022b. Stress and cancer: The mechanisms of immune dysregulation and management. Front Immunol 13, 1032294.

Louis, P., Flint, H.J., 2009. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett 294, 1-8.

Loupy, K.M., Arnold, M.R., Hassell, J.E., Jr., Lieb, M.W., Milton, L.N., Cler, K.E., Fox, J.H., Siebler, P.H., Schmidt, D., Noronha, S., Day, H.E.W., Lowry, C.A., 2019. Evidence that preimmunization with a heat-killed preparation of Mycobacterium

vaccae reduces corticotropin-releasing hormone mRNA expression in the extended amygdala in a fear-potentiated startle paradigm. Brain Behav Immun 77, 127-140.

Loupy, K.M., Cler, K.E., Marquart, B.M., Yifru, T.W., D'Angelo, H.M., Arnold, M.R., Elsayed, A.I., Gebert, M.J., Fierer, N., Fonken, L.K., Frank, M.G., Zambrano, C.A., Maier, S.F., Lowry, C.A., 2021. Comparing the effects of two different strains of mycobacteria, Mycobacterium vaccae NCTC 11659 and M. vaccae ATCC 15483, on stress-resilient behaviors and lipid-immune signaling in rats. Brain Behav Immun 91, 212-229.

Lowry, C.A., Hollis, J.H., de Vries, A., Pan, B., Brunet, L.R., Hunt, J.R., Paton, J.F., van Kampen, E., Knight, D.M., Evans, A.K., Rook, G.A., Lightman, S.L., 2007. Identification of an immune-responsive mesolimbocortical serotonergic system: potential role in regulation of emotional behavior. Neuroscience 146, 756-772.

Lu, B., Lin, L., Su, X., 2024. Global burden of depression or depressive symptoms in children and adolescents: A systematic review and meta-analysis. J Affect Disord 354, 553-562.

Lu, J., Chen, Y., Wang, Z., Cao, J., Dong, Y., 2021. The Role of the FOXO1/β(2)-AR/p-NF-κB p65 Pathway in the Development of Endometrial Stromal Cells in Pregnant Mice under Restraint Stress. Int J Mol Sci 22.

Lu, L.D., Stump, K.L., Seavey, M.M., 2010. Novel method of monitoring trace cytokines and activated STAT molecules in the paws of arthritic mice using multiplex bead technology. BMC Immunol 11, 55.

Ma, Y., Li, S.X., Zhou, R.Y., Deng, L.J., le He, W., Guo, L.L., Wang, L., Hao, J.H., Li, Y., Fang, M.F., Cao, Y.J., 2024. Geniposide improves depression-like behavior in prenatal stress male offspring through restoring HPA axis- and glucocorticoid receptor-associated dysfunction. Life Sci 340, 122434.

Maes, M., 1995. Evidence for an immune response in major depression: a review and hypothesis. Prog Neuropsychopharmacol Biol Psychiatry 19, 11-38.

Maes, M., 1999. Major depression and activation of the inflammatory response system. Adv Exp Med Biol 461, 25-46.

Maes, M., Lambrechts, J., Bosmans, E., Jacobs, J., Suy, E., Vandervorst, C., de Jonckheere, C., Minner, B., Raus, J., 1992. Evidence for a systemic immune activation during depression: results of leukocyte enumeration by flow cytometry in conjunction with monoclonal antibody staining. Psychol Med 22, 45-53.

Maes, M., Scharpé, S., Meltzer, H.Y., Bosmans, E., Suy, E., Calabrese, J., Cosyns, P., 1993. Relationships between interleukin-6 activity, acute phase proteins, and function of the hypothalamic-pituitary-adrenal axis in severe depression. Psychiatry Res 49, 11-27.

Magdeburg, R., Kessler, M., Boenninghoff, R., Keese, M., 2012. Imaging EGFR phosphorylation in intact human pancreatic carcinoma cells. In Vivo 26, 619-625.

Marteau, P., 2013. Butyrate-producing bacteria as pharmabiotics for inflammatory bowel disease. Gut 62, 1673.

Mawdsley, J.E., Rampton, D.S., 2005. Psychological stress in IBD: new insights into pathogenic and therapeutic implications. Gut 54, 1481-1491.

Mazzari, G., Lowry, C.A., Langgartner, D., Reber, S.O., 2023. Subcutaneous Mycobacterium vaccae ameliorates the effects of early life adversity alone or in combination with chronic stress during adulthood in male and female mice. Neurobiol Stress 26, 100568.

McBurney, M.I., Davis, C., Fraser, C.M., Schneeman, B.O., Huttenhower, C., Verbeke, K., Walter, J., Latulippe, M.E., 2019. Establishing What Constitutes a Healthy Human Gut Microbiome: State of the Science, Regulatory Considerations, and Future Directions. J Nutr 149, 1882-1895.

McDade, T.W., Hoke, M., Borja, J.B., Adair, L.S., Kuzawa, C., 2013. Do environments in infancy moderate the association between stress and inflammation in adulthood? Initial evidence from a birth cohort in the Philippines. Brain Behav Immun 31, 23-30.

McGregor, N.E., Murat, M., Elango, J., Poulton, I.J., Walker, E.C., Crimeen-Irwin, B., Ho, P.W.M., Gooi, J.H., Martin, T.J., Sims, N.A., 2019. IL-6 exhibits both cis- and trans-signaling in osteocytes and osteoblasts, but only trans-signaling promotes bone formation and osteoclastogenesis. J Biol Chem 294, 7850-7863.

McGuinness, A.J., Davis, J.A., Dawson, S.L., Loughman, A., Collier, F., O'Hely, M., Simpson, C.A., Green, J., Marx, W., Hair, C., Guest, G., Mohebbi, M., Berk, M., Stupart, D., Watters, D., Jacka, F.N., 2022. A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. Mol Psychiatry 27, 1920-1935.

Michopoulos, V., Powers, A., Gillespie, C.F., Ressler, K.J., Jovanovic, T., 2017. Inflammation in Fear- and Anxiety-Based Disorders: PTSD, GAD, and Beyond. Neuropsychopharmacology 42, 254-270.

Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry 65, 732-741.

Miller, A.H., Raison, C.L., 2016. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol 16, 22-34.

Mishra, A., Lai, G.C., Yao, L.J., Aung, T.T., Shental, N., Rotter-Maskowitz, A., Shepherdson, E., Singh, G.S.N., Pai, R., Shanti, A., Wong, R.M.M., Lee, A., Khyriem, C., Dutertre, C.A., Chakarov, S., Srinivasan, K.G., Shadan, N.B., Zhang, X.M., Khalilnezhad, S., Cottier, F., Tan, A.S.M., Low, G., Chen, P., Fan, Y., Hor, P.X., Lee, A.K.M., Choolani, M., Vermijlen, D., Sharma, A., Fuks, G., Straussman, R., Pavelka, N., Malleret, B., McGovern, N., Albani, S., Chan, J.K.Y., Ginhoux, F., 2021. Microbial exposure during early human development primes fetal immune cells. Cell 184, 3394-3409.e3320.

Mor, G., Cardenas, I., 2010. The immune system in pregnancy: a unique complexity. Am J Reprod Immunol 63, 425-433.

Moreau, J.L., 2002. Simulating the anhedonia symptom of depression in animals. Dialogues Clin Neurosci 4, 351-360.

Moura, C.A., Cagni, F.C., Costa, L.R.F., Tiago, P.R.F., Croyal, M., Aguesse, A., Reyes-Castro, L.A., Zambrano, E., Bolaños-Jiménez, F., Gavioli, E.C., 2022. Maternal Stress during Pregnancy in Mice Induces Sex-Dependent Behavioral Alterations in Offspring along with Impaired Serotonin and Kynurenine Pathways of Tryptophan Metabolism. Dev Neurosci 44, 603-614.

Moura, C.A., Oliveira, M.C., Costa, L.F., Tiago, P.R.F., Holanda, V.A.D., Lima, R.H., Cagni, F.C., Lobão-Soares, B., Bolaños-Jiménez, F., Gavioli, E.C., 2020. Prenatal restraint stress impairs recognition memory in adult male and female offspring. Acta Neuropsychiatr, 1-6.

Mueller, B.R., Bale, T.L., 2008. Sex-specific programming of offspring emotionality after stress early in pregnancy. J Neurosci 28, 9055-9065.

Munyoki, S.K., Goff, J.P., Reshke, A., Wilderoter, E., Mafarachisi, N., Kolobaric, A., Sheng, Y., Mullett, S.J., King, G.E., DeSchepper, J.D., Bookser, R.J., Castro, C.A., Gelhaus, S.L., Grizotte-Lake, M., Morrison, K.E., Zeleznik, A.J., Hand, T.W., Brieño-Enriquez, M.A., Jašarević, E., 2024. The microbiota extends the reproductive lifespan by safeguarding the ovarian reserve. bioRxiv, 2024.2009.2013.612929.

Murofushi, W., Mori, K., Murata, K., Yamaguchi, M., 2018. Functional development of olfactory tubercle domains during weaning period in mice. Sci Rep 8, 13204.

Musillo, C., Ajmone-Cat, M.A., De Simone, R., Tassinari, R., Maranghi, F., Tait, S., Samà, M., Giona, L., Pieroni, E.M., Alessi, R., Henning, T., Weber, D., Lippert, R.N., Pisanu, M.E., Chirico, M., Iorio, E., Fratini, F., Berry, A., Cirulli, F., 2025. Maternal stressors disrupt mouse placental proteome and fetal brain development in a sex-specific fashion through inflammation and oxidative stress. Mol Psychiatry.

Naik, S., Bouladoux, N., Wilhelm, C., Molloy, M.J., Salcedo, R., Kastenmuller, W., Deming, C., Quinones, M., Koo, L., Conlan, S., Spencer, S., Hall, J.A., Dzutsev, A., Kong, H., Campbell, D.J., Trinchieri, G., Segre, J.A., Belkaid, Y., 2012. Compartmentalized control of skin immunity by resident commensals. Science 337, 1115-1119.

Nederhof, E., Schmidt, M.V., 2012. Mismatch or cumulative stress: toward an integrated hypothesis of programming effects. Physiol Behav 106, 691-700.

Nogal, A., Valdes, A.M., Menni, C., 2021. The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. Gut Microbes 13, 1-24.

Nouioui, I., Brunet, L.R., Simpson, D., Klenk, H.P., Goodfellow, M., 2018. Description of a novel species of fast growing mycobacterium: Mycobacterium kyogaense sp. nov.,

a scotochromogenic strain received as Mycobacterium vaccae. Int J Syst Evol Microbiol 68, 3726-3734.

Nyuyki, K.D., Beiderbeck, D.I., Lukas, M., Neumann, I.D., Reber, S.O., 2012. Chronic subordinate colony housing (CSC) as a model of chronic psychosocial stress in male rats. PLoS One 7, e52371.

Orrù, G., Marzetti, F., Conversano, C., Vagheggini, G., Miccoli, M., Ciacchini, R., Panait, E., Gemignani, A., 2021. Secondary Traumatic Stress and Burnout in Healthcare Workers during COVID-19 Outbreak. Int J Environ Res Public Health 18.

Otaru, N., Kourouma, L., Pugin, B., Constancias, F., Braegger, C., Mansuy, I.M., Lacroix, C., 2024. Transgenerational effects of early life stress on the fecal microbiota in mice. Commun Biol 7, 670.

Oyola, M.G., Handa, R.J., 2017. Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity. Stress 20, 476-494.

Pace, T.W., Mletzko, T.C., Alagbe, O., Musselman, D.L., Nemeroff, C.B., Miller, A.H., Heim, C.M., 2006. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. Am J Psychiatry 163, 1630-1633.

Pace, T.W., Wingenfeld, K., Schmidt, I., Meinlschmidt, G., Hellhammer, D.H., Heim, C.M., 2012. Increased peripheral NF-κB pathway activity in women with childhood abuse-related posttraumatic stress disorder. Brain Behav Immun 26, 13-17.

Pardon, M., Gérardin, P., Joubert, C., Pérez-Diaz, F., Cohen-Salmon, C., 2000. Influence of prepartum chronic ultramild stress on maternal pup care behavior in mice. Biol Psychiatry 47, 858-863.

Pascuan, C.G., Rubinstein, M.R., Palumbo, M.L., Genaro, A.M., 2014. Prenatal stress induces up-regulation of glucocorticoid receptors on lymphoid cells modifying the T-cell response after acute stress exposure in the adult life. Physiol Behav 128, 141-147.

Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon, R.O., 3rd, Criqui, M., Fadl, Y.Y., Fortmann, S.P., Hong, Y., Myers, G.L., Rifai, N., Smith, S.C., Jr., Taubert, K., Tracy, R.P., Vinicor, F., 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 107, 499-511.

Peen, J., Schoevers, R.A., Beekman, A.T., Dekker, J., 2010. The current status of urban-rural differences in psychiatric disorders. Acta Psychiatrica Scandinavica 121, 84-93.

Peirce, J.M., Alviña, K., 2019. The role of inflammation and the gut microbiome in depression and anxiety. J Neurosci Res 97, 1223-1241.

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells,

D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Würbel, H., 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18, e3000410.

Pereg, D., Tirosh, A., Lishner, M., Goldberg, A., Shochat, T., Confino-Cohen, R., 2008. Prevalence of asthma in a large group of Israeli adolescents: influence of country of birth and age at migration. Allergy 63, 1040-1045.

Peters, S., Grunwald, N., Rümmele, P., Endlicher, E., Lechner, A., Neumann, I.D., Obermeier, F., Reber, S.O., 2012. Chronic psychosocial stress increases the risk for inflammation-related colon carcinogenesis in male mice. Stress 15, 403-415.

Peterson, C.T., Perez Santiago, J., Iablokov, S.N., Chopra, D., Rodionov, D.A., Peterson, S.N., 2022. Short-Chain Fatty Acids Modulate Healthy Gut Microbiota Composition and Functional Potential. Curr Microbiol 79, 128.

Pfefferle, P.I., Keber, C.U., Cohen, R.M., Garn, H., 2021. The Hygiene Hypothesis - Learning From but Not Living in the Past. Front Immunol 12, 635935.

Piao, X., Xie, J., Managi, S., 2024. Continuous worsening of population emotional stress globally: universality and variations. BMC Public Health 24, 3576.

Plant, D.T., Pawlby, S., Sharp, D., Zunszain, P.A., Pariante, C.M., 2016. Prenatal maternal depression is associated with offspring inflammation at 25 years: a prospective longitudinal cohort study. Transl Psychiatry 6, e936.

Podolsky, D.K., 2002. Inflammatory bowel disease. N Engl J Med 347, 417-429.

Qin, X., Zhang, M., Chen, S., Tang, Y., Cui, J., Ding, G., 2024. Short-chain fatty acids in fetal development and metabolism. Trends Mol Med.

Qing, L., Qian, X., Zhu, H., Wang, J., Sun, J., Jin, Z., Tang, X., Zhao, Y., Wang, G., Zhao, J., Chen, W., Tian, P., 2025. Maternal-infant probiotic transmission mitigates early-life stress-induced autism in mice. Gut Microbes 17, 2456584.

Quan, N., Avitsur, R., Stark, J.L., He, L., Lai, W., Dhabhar, F., Sheridan, J.F., 2003. Molecular mechanisms of glucocorticoid resistance in splenocytes of socially stressed male mice. J Neuroimmunol 137, 51-58.

Raison, C.L., Lowry, C.A., Rook, G.A., 2010. Inflammation, sanitation, and consternation: loss of contact with coevolved, tolerogenic microorganisms and the pathophysiology and treatment of major depression. Arch Gen Psychiatry 67, 1211-1224.

Raison, C.L., Miller, A.H., 2003. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am J Psychiatry 160, 1554-1565.

Ramanan, D., Sefik, E., Galván-Peña, S., Wu, M., Yang, L., Yang, Z., Kostic, A., Golovkina, T.V., Kasper, D.L., Mathis, D., Benoist, C., 2020. An Immunologic Mode of

Multigenerational Transmission Governs a Gut Treg Setpoint. Cell 181, 1276-1290.e1213.

Rao, R.T., Androulakis, I.P., 2017. Modeling the Sex Differences and Interindividual Variability in the Activity of the Hypothalamic-Pituitary-Adrenal Axis. Endocrinology 158, 4017-4037.

Ratsika, A., Codagnone, M.C., O'Mahony, S., Stanton, C., Cryan, J.F., 2021. Priming for Life: Early Life Nutrition and the Microbiota-Gut-Brain Axis. Nutrients 13.

Reber, S.O., 2012. Stress and animal models of inflammatory bowel disease--an update on the role of the hypothalamo-pituitary-adrenal axis. Psychoneuroendocrinology 37, 1-19.

Reber, S.O., Birkeneder, L., Veenema, A.H., Obermeier, F., Falk, W., Straub, R.H., Neumann, I.D., 2007. Adrenal insufficiency and colonic inflammation after a novel chronic psycho-social stress paradigm in mice: implications and mechanisms. Endocrinology 148, 670-682.

Reber, S.O., Langgartner, D., Foertsch, S., Postolache, T.T., Brenner, L.A., Guendel, H., Lowry, C.A., 2016a. Chronic subordinate colony housing paradigm: A mouse model for mechanisms of PTSD vulnerability, targeted prevention, and treatment-2016 Curt Richter Award Paper. Psychoneuroendocrinology 74, 221-230.

Reber, S.O., Neumann, I.D., 2008. Defensive behavioral strategies and enhanced state anxiety during chronic subordinate colony housing are accompanied by reduced hypothalamic vasopressin, but not oxytocin, expression. Ann N Y Acad Sci 1148, 184-195.

Reber, S.O., Obermeier, F., Straub, R.H., Veenema, A.H., Neumann, I.D., 2008. Aggravation of DSS-induced colitis after chronic subordinate colony (CSC) housing is partially mediated by adrenal mechanisms. Stress 11, 225-234.

Reber, S.O., Peters, S., Slattery, D.A., Hofmann, C., Schölmerich, J., Neumann, I.D., Obermeier, F., 2011. Mucosal immunosuppression and epithelial barrier defects are key events in murine psychosocial stress-induced colitis. Brain Behav Immun 25, 1153-1161.

Reber, S.O., Siebler, P.H., Donner, N.C., Morton, J.T., Smith, D.G., Kopelman, J.M., Lowe, K.R., Wheeler, K.J., Fox, J.H., Hassell, J.E., Jr., Greenwood, B.N., Jansch, C., Lechner, A., Schmidt, D., Uschold-Schmidt, N., Fuchsl, A.M., Langgartner, D., Walker, F.R., Hale, M.W., Lopez Perez, G., Van Treuren, W., Gonzalez, A., Halweg-Edwards, A.L., Fleshner, M., Raison, C.L., Rook, G.A., Peddada, S.D., Knight, R., Lowry, C.A., 2016b. Immunization with a heat-killed preparation of the environmental bacterium Mycobacterium vaccae promotes stress resilience in mice. Proc Natl Acad Sci U S A 113, E3130-3139.

Reul, J.M., de Kloet, E.R., 1985. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117, 2505-2511.

Richter, J.G., Muth, T., Li, J., Brinks, R., Chehab, G., Koch, T., Siegrist, J., Angerer, P., Huscher, D., Schneider, M., 2018. Elevated Psychosocial Stress at Work in Patients with Systemic Lupus Erythematosus and Rheumatoid Arthritis. J Rheumatol 45, 227-234.

Riedler, J., Braun-Fahrländer, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., Carr, D., Schierl, R., Nowak, D., von Mutius, E., 2001. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. The Lancet 358, 1129-1133.

Rissing, J.P., Buxton, T.B., Edmondson, H.T., 1979. Detection of specific IgG antibody in sera from patients infected with Bacteroides fragilis by enzyme-linked immunosorbent assay. J Infect Dis 140, 994-998.

Robertson, D.A., Ray, J., Diamond, I., Edwards, J.G., 1989. Personality profile and affective state of patients with inflammatory bowel disease. Gut 30, 623-626.

Robertson, S.J., Lemire, P., Maughan, H., Goethel, A., Turpin, W., Bedrani, L., Guttman, D.S., Croitoru, K., Girardin, S.E., Philpott, D.J., 2019. Comparison of Co-housing and Littermate Methods for Microbiota Standardization in Mouse Models. Cell Rep 27, 1910-1919.e1912.

Robinson, M., Carter, K.W., Pennell, C.E., Jacoby, P., Moore, H.C., Zubrick, S.R., Burgner, D., 2021. Maternal prenatal stress exposure and sex-specific risk of severe infection in offspring. PLoS One 16, e0245747.

Rodríguez-Güell, E., Agustí, G., Corominas, M., Cardona, P.J., Casals, I., Parella, T., Sempere, M.A., Luquin, M., Julián, E., 2006. The production of a new extracellular putative long-chain saturated polyester by smooth variants of Mycobacterium vaccae interferes with Th1-cytokine production. Antonie Van Leeuwenhoek 90, 93-108.

Rohleder, N., 2014. Stimulation of systemic low-grade inflammation by psychosocial stress. Psychosom Med 76, 181-189.

Rook, G.A., 2013. Regulation of the immune system by biodiversity from the natural environment: an ecosystem service essential to health. Proc Natl Acad Sci U S A 110, 18360-18367.

Rook, G.A., Adams, V., Hunt, J., Palmer, R., Martinelli, R., Brunet, L.R., 2004. Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. Springer Semin Immunopathol 25, 237-255.

Rook, G.A., Lowry, C.A., Raison, C.L., 2013a. Microbial 'Old Friends', immunoregulation and stress resilience. Evolution, Medicine, and Public Health 2013, 46-64.

Rook, G.A., Raison, C.L., Lowry, C.A., 2012. Can we vaccinate against depression? Drug Discov Today 17, 451-458.

Rook, G.A., Raison, C.L., Lowry, C.A., 2013b. Childhood microbial experience, immunoregulation, inflammation and adult susceptibility to psychosocial stressors and

depression in rich and poor countries. Evolution, Medicine, and Public Health 2013, 14-17.

Rook, G.A., Raison, C.L., Lowry, C.A., 2014. Microbial 'old friends', immunoregulation and socioeconomic status. Clin Exp Immunol 177, 1-12.

Rook, G.A.W., 2023. The old friends hypothesis: evolution, immunoregulation and essential microbial inputs. Front Allergy 4, 1220481.

Rosenberg, R., Vinker, S., Zakut, H., Kizner, F., Nakar, S., Kitai, E., 1999. An unusually high prevalence of asthma in Ethiopian immigrants to Israel. Fam Med 31, 276-279.

Rottem, M., Szyper-Kravitz, M., Shoenfeld, Y., 2005. Atopy and asthma in migrants. Int Arch Allergy Immunol 136, 198-204.

Round, J.L., Lee, S.M., Li, J., Tran, G., Jabri, B., Chatila, T.A., Mazmanian, S.K., 2011. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 332, 974-977.

Round, J.L., Mazmanian, S.K., 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9, 313-323.

Ruschak, I., Montesó-Curto, P., Rosselló, L., Aguilar Martín, C., Sánchez-Montesó, L., Toussaint, L., 2023. Fibromyalgia Syndrome Pain in Men and Women: A Scoping Review. Healthcare (Basel) 11.

Sachs, B.D., Ni, J.R., Caron, M.G., 2014. Sex differences in response to chronic mild stress and congenital serotonin deficiency. Psychoneuroendocrinology 40, 123-129.

Sanchez, K., Darling, J.S., Kakkar, R., Wu, S.L., Zentay, A., Lowry, C.A., Fonken, L.K., 2022. Mycobacterium vaccae immunization in rats ameliorates features of age-associated microglia activation in the amygdala and hippocampus. Sci Rep 12, 2165.

Santarelli, S., Zimmermann, C., Kalideris, G., Lesuis, S.L., Arloth, J., Uribe, A., Dournes, C., Balsevich, G., Hartmann, J., Masana, M., Binder, E.B., Spengler, D., Schmidt, M.V., 2017. An adverse early life environment can enhance stress resilience in adulthood. Psychoneuroendocrinology 78, 213-221.

Savino, F., Galliano, I., Garro, M., Savino, A., Daprà, V., Montanari, P., Bergallo, M., 2018a. Regulatory T cells and Toll-like receptor 2 and 4 mRNA expression in infants with colic treated with Lactobacillus reuteri DSM17938. Benef Microbes 9, 917-925.

Savino, F., Garro, M., Montanari, P., Galliano, I., Bergallo, M., 2018b. Crying Time and RORγ/FOXP3 Expression in Lactobacillus reuteri DSM17938-Treated Infants with Colic: A Randomized Trial. J Pediatr 192, 171-177.e171.

Saxena, Y., Routh, S., Mukhopadhaya, A., 2021. Immunoporosis: Role of Innate Immune Cells in Osteoporosis. Front Immunol 12, 687037.

Schaub, B., Liu, J., Höppler, S., Schleich, I., Huehn, J., Olek, S., Wieczorek, G., Illi, S., von Mutius, E., 2009. Maternal farm exposure modulates neonatal immune mechanisms

through regulatory T cells. Journal of Allergy and Clinical Immunology 123, 774-782.e775.

Schiele, J., Mazzari, G., Struck, A., Bailer, Y., Langgartner, D., Reber, S.O., 2024. Chronic sensory contact with subordinated conspecifics promotes splenic glucocorticoid resistance in experimentally wounded C57BL/6N male mice. Sci Rep 14, 10867.

Schiele, J., Tsai, P.L., Ernst, G., Blank, C., Beck, S., Schimmele, T., Burth, E., Gebauer, D., Lowry, C.A., Noschka, R., Langgartner, D., Stenger, S., Haffner-Luntzer, M., Slattery, D.A., Reber, S.O., 2025. Mycobacterium vaccae ATCC 15483(T) administered to nulliparous C57BL/6N mice prior to mating protects their male offspring against the negative consequences of prenatal stress. Brain Behav Immun 131, 106169.

Schiele, J., Tsai, P.L., Schimmele, T., Beck, S., Meyer, M., Mannes, M., Desmond, L.W., Noschka, R., Huber-Lang, M., Haffner-Luntzer, M., Jarczok, M.N., Lowry, C.A., Reif, A., Langgartner, D., Stenger, S., Slattery, D.A., Reber, S.O., 2026. Microbial Legacy: Mycobacterium vaccae ATCC 15483(T) intergenerationally diversifies the microbiome and enhances stress resilience in male mice. Mol Psychiatry.

Schimmele, T., Langgartner, D., Gebauer, D., Mazzari, G., Probst, J., Weingast, G., Ignatius, A., Tabacco, G., Naciu, A.M., Messina, M.V., Palermo, A., Reber, S.O., Haffner-Luntzer, M., 2025. Early life adversity promotes a milieu in favor of catabolic bone turnover in females: Mycobacterium vaccae NCTC 11659 proofs protective in preclinical studies. Brain Behav Immun.

Schmidt, D., Peterlik, D., Reber, S.O., Lechner, A., Männel, D.N., 2016. Induction of Suppressor Cells and Increased Tumor Growth following Chronic Psychosocial Stress in Male Mice. PLoS One 11, e0159059.

Schmidt, M., Lapert, F., Brandwein, C., Deuschle, M., Kasperk, C., Grimsley, J.M., Gass, P., 2017. Prenatal stress changes courtship vocalizations and bone mineral density in mice. Psychoneuroendocrinology 75, 203-212.

Schmidt, M.V., 2011. Animal models for depression and the mismatch hypothesis of disease. Psychoneuroendocrinology 36, 330-338.

Schmidt, M.V., Scharf, S.H., Liebl, C., Harbich, D., Mayer, B., Holsboer, F., Müller, M.B., 2010. A novel chronic social stress paradigm in female mice. Horm Behav 57, 415-420.

Schmidt, M.V., Sterlemann, V., Ganea, K., Liebl, C., Alam, S., Harbich, D., Greetfeld, M., Uhr, M., Holsboer, F., Müller, M.B., 2007. Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. Psychoneuroendocrinology 32, 417-429.

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3, 1101-1108.

Seckl, J.R., Cleasby, M., Nyirenda, M.J., 2000. Glucocorticoids, 11beta-hydroxysteroid dehydrogenase, and fetal programming. Kidney Int 57, 1412-1417.

Sheridan, J.F., Stark, J.L., Avitsur, R., Padgett, D.A., 2000. Social disruption, immunity, and susceptibility to viral infection. Role of glucocorticoid insensitivity and NGF. Ann N Y Acad Sci 917, 894-905.

Sherwin, E., Rea, K., Dinan, T.G., Cryan, J.F., 2016. A gut (microbiome) feeling about the brain. Curr Opin Gastroenterol 32, 96-102.

Siebler, P.H., Heinze, J.D., Kienzle, D.M., Hale, M.W., Lukkes, J.L., Donner, N.C., Kopelman, J.M., Rodriguez, O.A., Lowry, C.A., 2018. Acute Administration of the Nonpathogenic, Saprophytic Bacterium, Mycobacterium vaccae, Induces Activation of Serotonergic Neurons in the Dorsal Raphe Nucleus and Antidepressant-Like Behavior in Association with Mild Hypothermia. Cell Mol Neurobiol 38, 289-304.

Singewald, G.M., Nguyen, N.K., Neumann, I.D., Singewald, N., Reber, S.O., 2009. Effect of chronic psychosocial stress-induced by subordinate colony (CSC) housing on brain neuronal activity patterns in mice. Stress 12, 58-69.

Slattery, D.A., Uschold, N., Magoni, M., Bär, J., Popoli, M., Neumann, I.D., Reber, S.O., 2012. Behavioural consequences of two chronic psychosocial stress paradigms: anxiety without depression. Psychoneuroendocrinology 37, 702-714.

Smith, D.G., Martinelli, R., Besra, G.S., Illarionov, P.A., Szatmari, I., Brazda, P., Allen, M.A., Xu, W., Wang, X., Nagy, L., Dowell, R.D., Rook, G.A.W., Rosa Brunet, L., Lowry, C.A., 2019. Identification and characterization of a novel anti-inflammatory lipid isolated from Mycobacterium vaccae, a soil-derived bacterium with immunoregulatory and stress resilience properties. Psychopharmacology (Berl) 236, 1653-1670.

Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly, Y.M., Glickman, J.N., Garrett, W.S., 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 341, 569-573.

Smith, Z.Z., Kubiak, R.A., Arnold, M.R., Loupy, K.M., Taylor, J.A., Crist, T.G., Bernier, A.E., D'Angelo, H.M., Heinze, J.D., Lowry, C.A., Barth, D.S., 2020. Effects of immunization with heat-killed Mycobacterium vaccae on autism spectrum disorder-like behavior and epileptogenesis in a rat model of comorbid autism and epilepsy. Brain Behav Immun 88, 763-780.

Söderström, U., Aman, J., Hjern, A., 2012. Being born in Sweden increases the risk for type 1 diabetes - a study of migration of children to Sweden as a natural experiment. Acta Paediatr 101, 73-77.

Sommershof, A., Aichinger, H., Engler, H., Adenauer, H., Catani, C., Boneberg, E.M., Elbert, T., Groettrup, M., Kolassa, I.T., 2009. Substantial reduction of naïve and regulatory T cells following traumatic stress. Brain Behav Immun 23, 1117-1124.

Spinillo, A., Iacobone, A.D., Calvino, I.G., Alberi, I., Gardella, B., 2014. The role of the placenta in feto-neonatal infections. Early Hum Dev 90 Suppl 1, S7-9.

Spitzer, C., Barnow, S., Völzke, H., Wallaschofski, H., John, U., Freyberger, H.J., Löwe, B., Grabe, H.J., 2010. Association of posttraumatic stress disorder with low-grade elevation of C-reactive protein: evidence from the general population. J Psychiatr Res 44, 15-21.

Spragge, F., Bakkeren, E., Jahn, M.T., E, B.N.A., Pearson, C.F., Wang, X., Pankhurst, L., Cunrath, O., Foster, K.R., 2023. Microbiome diversity protects against pathogens by nutrient blocking. Science 382, eadj3502.

Stanford, J., Stanford, C., 2012. Mycobacteria and their world. Int J Mycobacteriol 1, 3-12.

Stanford, J., Stanford, C., Grange, J., 2004. Immunotherapy with Mycobacterium vaccae in the treatment of tuberculosis. Front Biosci 9, 1701-1719.

Stanford, J., Stanford, C., Stansby, G., Bottasso, O., Bahr, G., Grange, J., 2009. The common mycobacterial antigens and their importance in the treatment of disease. Curr Pharm Des 15, 1248-1260.

Stanford, J.L., Paul, R.C., 1973. A preliminary report on some studies of environmental mycobacteria. Ann Soc Belg Med Trop 53, 389-393.

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., Müller, M.B., Schmidt, M.V., 2008. Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: implications for stress-related disorders. Horm Behav 53, 386-394.

Strachan, D.P., 1989. Hay fever, hygiene, and household size. Bmj 299, 1259-1260.

Strachan, D.P., 2000. Family size, infection and atopy: the first decade of the "hygiene hypothesis". Thorax 55 Suppl 1, S2-10.

Suenaga, T., Yukie, M., Gao, S., Nakahara, D., 2012. Sex-specific effects of prenatal stress on neuronal development in the medial prefrontal cortex and the hippocampus. Neuroreport 23, 430-435.

Sun, Y., Xie, R., Li, L., Jin, G., Zhou, B., Huang, H., Li, M., Yang, Y., Liu, X., Cao, X., Wang, B., Liu, W., Jiang, K., Cao, H., 2021. Prenatal Maternal Stress Exacerbates Experimental Colitis of Offspring in Adulthood. Front Immunol 12, 700995.

Takahashi, A., Chung, J.R., Zhang, S., Zhang, H., Grossman, Y., Aleyasin, H., Flanigan, M.E., Pfau, M.L., Menard, C., Dumitriu, D., Hodes, G.E., McEwen, B.S., Nestler, E.J., Han, M.H., Russo, S.J., 2017. Establishment of a repeated social defeat stress model in female mice. Sci Rep 7, 12838.

Tandon, D., Haque, M.M., Gote, M., Jain, M., Bhaduri, A., Dubey, A.K., Mande, S.S., 2019. A prospective randomized, double-blind, placebo-controlled, dose-response relationship study to investigate efficacy of fructo-oligosaccharides (FOS) on human gut microflora. Sci Rep 9, 5473.

Taylor, A.G., Fischer-White, T.G., Anderson, J.G., Adelstein, K.E., Murugesan, M., Lewis, J.E., Scott, M.M., Gaykema, R.P., Goehler, L.E., 2016. Stress, Inflammation and Pain:

A Potential Role for Monocytes in Fibromyalgia-related Symptom Severity. Stress Health 32, 503-513.

Tofani, G.S.S., Leigh, S.J., Gheorghe, C.E., Bastiaanssen, T.F.S., Wilmes, L., Sen, P., Clarke, G., Cryan, J.F., 2025. Gut microbiota regulates stress responsivity via the circadian system. Cell Metab 37, 138-153.e135.

Tschaffon-Müller, M.E.A., Kempter, E., Steppe, L., Kupfer, S., Kuhn, M.R., Gebhard, F., Pankratz, C., Kalbitz, M., Schütze, K., Gündel, H., Kaleck, N., Strauß, G., Vacher, J., Ichinose, H., Weimer, K., Ignatius, A., Haffner-Luntzer, M., Reber, S.O., 2023. Neutrophil-derived catecholamines mediate negative stress effects on bone. Nat Commun 14, 3262.

Tshikudi, D.M., Bernstein, C.N., Mishra, S., Ghia, J.E., Armstrong, H.K., 2025. Influence of biological sex in inflammatory bowel diseases. Nat Rev Gastroenterol Hepatol 22, 415-437.

Turner, M.L., Healey, G.D., Sheldon, I.M., 2012. Immunity and inflammation in the uterus. Reprod Domest Anim 47 Suppl 4, 402-409.

Tusa, B.S., Alati, R., Ayano, G., Betts, K., Weldesenbet, A.B., Dachew, B., 2025. Maternal pre- and perinatal depression and the risk of autism spectrum disorders in offspring: systematic review and meta-analysis. BJPsych Open 11, e117.

UK Health Security Agency, 1973. Culutre Collections Bacteria Collection: *Mycobacterium vaccae*.

Undén, A.L., Orth-Gomér, K., Elofsson, S., 1991. Cardiovascular effects of social support in the work place: twenty-four-hour ECG monitoring of men and women. Psychosom Med 53, 50-60.

Uschold-Schmidt, N., Nyuyki, K.D., Füchsl, A.M., Neumann, I.D., Reber, S.O., 2012. Chronic psychosocial stress results in sensitization of the HPA axis to acute heterotypic stressors despite a reduction of adrenal in vitro ACTH responsiveness. Psychoneuroendocrinology 37, 1676-1687.

Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E.F., Wang, J., Tito, R.Y., Schiweck, C., Kurilshikov, A., Joossens, M., Wijmenga, C., Claes, S., Van Oudenhove, L., Zhernakova, A., Vieira-Silva, S., Raes, J., 2019. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol 4, 623-632.

Van den Bergh, B.R.H., van den Heuvel, M.I., Lahti, M., Braeken, M., de Rooij, S.R., Entringer, S., Hoyer, D., Roseboom, T., Räikkönen, K., King, S., Schwab, M., 2020. Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. Neurosci Biobehav Rev 117, 26-64.

van Doeselaar, L., Yang, H., Bordes, J., Brix, L., Engelhardt, C., Tang, F., Schmidt, M.V., 2021. Chronic social defeat stress in female mice leads to sex-specific behavioral and neuroendocrine effects. Stress 24, 168-180.

Vazquez-Torres, A., Jones-Carson, J., Bäumler, A.J., Falkow, S., Valdivia, R., Brown, W., Le, M., Berggren, R., Parks, W.T., Fang, F.C., 1999. Extraintestinal dissemination of Salmonella by CD18-expressing phagocytes. Nature 401, 804-808.

Veenema, A.H., Sijtsma, B., Koolhaas, J.M., de Kloet, E.R., 2005. The stress response to sensory contact in mice: genotype effect of the stimulus animal. Psychoneuroendocrinology 30, 550-557.

Vermeire, S., Van Assche, G., Rutgeerts, P., 2004. C-reactive protein as a marker for inflammatory bowel disease. Inflamm Bowel Dis 10, 661-665.

Voigt, R.M., Zalta, A.K., Raeisi, S., Zhang, L., Brown, J.M., Forsyth, C.B., Boley, R.A., Held, P., Pollack, M.H., Keshavarzian, A., 2022. Abnormal intestinal milieu in posttraumatic stress disorder is not impacted by treatment that improves symptoms. Am J Physiol Gastrointest Liver Physiol 323, G61-g70.

von Känel, R., Begré, S., Abbas, C.C., Saner, H., Gander, M.L., Schmid, J.P., 2010. Inflammatory biomarkers in patients with posttraumatic stress disorder caused by myocardial infarction and the role of depressive symptoms. Neuroimmunomodulation 17, 39-46.

Vujkovic-Cvijin, I., Welles, H.C., Ha, C.W.Y., Huq, L., Mistry, S., Brenchley, J.M., Trinchieri, G., Devkota, S., Belkaid, Y., 2022. The systemic anti-microbiota IgG repertoire can identify gut bacteria that translocate across gut barrier surfaces. Sci Transl Med 14, eabl3927.

Waldbaum, J.D.H., Xhumari, J., Akinsuyi, O.S., Arjmandi, B., Anton, S., Roesch, L.F.W., 2023. Association between Dysbiosis in the Gut Microbiota of Primary Osteoporosis Patients and Bone Loss. Aging Dis 14, 2081-2095.

Walk, S.T., Blum, A.M., Ewing, S.A., Weinstock, J.V., Young, V.B., 2010. Alteration of the murine gut microbiota during infection with the parasitic helminth Heligmosomoides polygyrus. Inflamm Bowel Dis 16, 1841-1849.

Walker, R.L., Vlamakis, H., Lee, J.W.J., Besse, L.A., Xanthakis, V., Vasan, R.S., Shaw, S.Y., Xavier, R.J., 2021. Population study of the gut microbiome: associations with diet, lifestyle, and cardiometabolic disease. Genome Med 13, 188.

Walton, C., King, R., Rechtman, L., Kaye, W., Leray, E., Marrie, R.A., Robertson, N., La Rocca, N., Uitdehaag, B., van der Mei, I., Wallin, M., Helme, A., Angood Napier, C., Rijke, N., Baneke, P., 2020. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. Mult Scler 26, 1816-1821.

Wang, C.C., Rook, G.A., 1998. Inhibition of an established allergic response to ovalbumin in BALB/c mice by killed Mycobacterium vaccae. Immunology 93, 307-313.

Wang, K., Wang, S., Chen, Y., Lu, X., Wang, D., Zhang, Y., Pan, W., Zhou, C., Zou, D., 2024a. Causal relationship between gut microbiota and risk of gastroesophageal reflux disease: a genetic correlation and bidirectional Mendelian randomization study. Frontiers in Immunology Volume 15 - 2024.

Wang, S., Dong, Z., Wan, X., 2024b. Global, regional, and national burden of inflammatory bowel disease and its associated anemia, 1990 to 2019 and predictions to 2050: An analysis of the global burden of disease study 2019. Autoimmun Rev 23, 103498.

Welsh, A., Hammad, M., Piña, I.L., Kulinski, J., 2024. Obesity and cardiovascular health. Eur J Prev Cardiol 31, 1026-1035.

Wexler, H.M., 2007. Bacteroides: the good, the bad, and the nitty-gritty. Clin Microbiol Rev 20, 593-621.

Wolfe, N.D., Dunavan, C.P., Diamond, J., 2007. Origins of major human infectious diseases. Nature 447, 279-283.

Woolcock, A.J., Peat, J.K., 1997. Evidence for the increase in asthma worldwide. Ciba Found Symp 206, 122-134; discussion 134-129, 157-129.

Wright, R.J., Finn, P., Contreras, J.P., Cohen, S., Wright, R.O., Staudenmayer, J., Wand, M., Perkins, D., Weiss, S.T., Gold, D.R., 2004. Chronic caregiver stress and IgE expression, allergen-induced proliferation, and cytokine profiles in a birth cohort predisposed to atopy. J Allergy Clin Immunol 113, 1051-1057.

Wu, W.C., Hu, D.N., Gao, H.X., Chen, M., Wang, D., Rosen, R., McCormick, S.A., 2010. Subtoxic levels hydrogen peroxide-induced production of interleukin-6 by retinal pigment epithelial cells. Mol Vis 16, 1864-1873.

Xu, C., Miao, L., Turner, D., DeRubeis, R., 2023a. Urbanicity and depression: A global meta-analysis. J Affect Disord 340, 299-311.

Xu, D., Zhou, S., Liu, Y., Scott, A.L., Yang, J., Wan, F., 2024. Complement in breast milk modifies offspring gut microbiota to promote infant health. Cell 187, 750-763.e720.

Xu, J., Liu, N., Polemiti, E., Garcia-Mondragon, L., Tang, J., Liu, X., Lett, T., Yu, L., Nöthen, M.M., Feng, J., Yu, C., Marquand, A., Schumann, G., 2023b. Effects of urban living environments on mental health in adults. Nat Med 29, 1456-1467.

Yamamura, M., Wang, X.H., Ohmen, J.D., Uyemura, K., Rea, T.H., Bloom, B.R., Modlin, R.L., 1992. Cytokine patterns of immunologically mediated tissue damage. J Immunol 149, 1470-1475.

Yehuda, R., 2002. Post-traumatic stress disorder. N Engl J Med 346, 108-114.

Yohn, C.N., Ashamalla, S.A., Bokka, L., Gergues, M.M., Garino, A., Samuels, B.A., 2019. Social instability is an effective chronic stress paradigm for both male and female mice. Neuropharmacology 160, 107780.

Yotova, A.Y., Li, L.L., O'Leary, A., Tegeder, I., Reif, A., Courtney, M.J., Slattery, D.A., Freudenberg, F., 2024. Synaptic proteome perturbations after maternal immune activation: Identification of embryonic and adult hippocampal changes. Brain Behav Immun 121, 351-364.

Zaiss, M.M., Jones, R.M., Schett, G., Pacifici, R., 2019. The gut-bone axis: how bacterial metabolites bridge the distance. J Clin Invest 129, 3018-3028.

Zanker, B., Walz, G., Wieder, K.J., Strom, T.B., 1990. Evidence that glucocorticosteroids block expression of the human interleukin-6 gene by accessory cells. Transplantation 49, 183-185.

Zeng, M.Y., Cisalpino, D., Varadarajan, S., Hellman, J., Warren, H.S., Cascalho, M., Inohara, N., Núñez, G., 2016. Gut Microbiota-Induced Immunoglobulin G Controls Systemic Infection by Symbiotic Bacteria and Pathogens. Immunity 44, 647-658.

Zhang, K., Kan, C., Han, F., Zhang, J., Ding, C., Guo, Z., Huang, N., Zhang, Y., Hou, N., Sun, X., 2023. Global, Regional, and National Epidemiology of Diabetes in Children From 1990 to 2019. JAMA Pediatr 177, 837-846.

Zhang, Z., Li, N., Chen, R., Lee, T., Gao, Y., Yuan, Z., Nie, Y., Sun, T., 2021. Prenatal stress leads to deficits in brain development, mood related behaviors and gut microbiota in offspring. Neurobiol Stress 15, 100333.

Zhong, C., Wang, Q., He, Y., Zhao, X., Wang, Y., He, L., Wei, H., Tao, X., 2023. Maternal supplementation with human milk-derived Lactiplantibacillus plantarum WLPL04 affects the immunity and gut microbiota of offspring rats. Food Funct 14, 5326-5341.

Zijlmans, M.A., Korpela, K., Riksen-Walraven, J.M., de Vos, W.M., de Weerth, C., 2015. Maternal prenatal stress is associated with the infant intestinal microbiota. Psychoneuroendocrinology 53, 233-245.

Zoubovsky, S.P., Hoseus, S., Tumukuntala, S., Schulkin, J.O., Williams, M.T., Vorhees, C.V., Muglia, L.J., 2020. Chronic psychosocial stress during pregnancy affects maternal behavior and neuroendocrine function and modulates hypothalamic CRH and nuclear steroid receptor expression. Transl Psychiatry 10, 6.

Zoubovsky, S.P., Williams, M.T., Hoseus, S., Tumukuntala, S., Riesenberg, A., Schulkin, J., Vorhees, C.V., Campbell, K., Lim, H.W., Muglia, L.J., 2022. Neurobehavioral abnormalities following prenatal psychosocial stress are differentially modulated by maternal environment. Transl Psychiatry 12, 22.

Zuany-Amorim, C., Sawicka, E., Manlius, C., Le Moine, A., Brunet, L.R., Kemeny, D.M., Bowen, G., Rook, G., Walker, C., 2002. Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. Nat Med 8, 625-629.

Zucker, I., Beery, A.K., 2010. Males still dominate animal studies. Nature 465, 690.

During the preparation of this work I used ChatGPT Plus in order to check the grammar of individual sentences. After using this tool, I reviewed the sentences and edited them as needed. I take full responsibility for the grammatical style of this thesis.

List of Publications

Schiele J[*], Tsai PL[*], Schimmele T, Beck S, Meyer M, Mannes M, Desmond LW, Noschka R, Huber-Lang M, Haffner-Luntzer M, Jarczok MN, Lowry CA, Reif A, Langgartner D, Stenger S, Slattery DA[#], Reber SO[#], **2026.** Microbial Legacy: *Mycobacterium vaccae* ATCC 15483^{T} intergenerationally diversifies the microbiome and enhances stress resilience in male mice. *Molecular Psychiatry*. doi: 10.1038/s41380-026-03638-9.
*both first authors contributed equally, [#] both last authors contributed equally.

Molecular Psychiatry allows the reuse of this article in a thesis/dissertation as full article in both printed and electronic format (licensed under CC BY 4.0).

Schiele J[*], Tsai PL[*], Ernst G, Blank C, Beck S, Schimmele T, Burth E, Gebauer D, Lowry CA, Noschka R, Langgartner D, Stenger S, Haffner-Luntzer M, Slattery DA[*], Reber SO[*], **2026 (Epub 2025).** *Mycobacterium vaccae* ATCC 15483T administered to nulliparous C57BL/6N mice prior to mating protects their male offspring against the negative consequences of prenatal stress. *Brain, Behavior, and Immunity* 131:106169.
doi: 10.1016/j.bbi.2025.106169.
*both first authors contributed equally, [#] both last authors contributed equally.

Brain, Behavior, and Immunity allows the reuse of this article in a thesis/dissertation as full article in both printed and electronic format (licensed under CC BY 4.0).

Schiele J, Mazzari G, Struck A, Bailer Y, Langgartner D, Reber SO, **2024**
Chronic sensory contact with subordinated conspecifics promotes splenic glucocorticoid resistance in experimentally wounded C57BL/6N male mice. *Scientific Reports* 14(1):10867. doi: 10.1038/s41598-024-61581-7

Scientific Reports allows the reuse of this article in a thesis/dissertation as full article in both printed and electronic format (licensed under CC BY 4.0).

Acknowledgments

This fascinating, complex, challenging, however successful project would not have been possible without the support and help of many people. It is therefore very important to me to thank them, in the hope that they will all receive my appreciation and gratitude either directly or indirectly.

First and foremost, I would like to thank Prof. Harald Gündel for the opportunity to become a PhD student at the Department of Psychosomatic Medicine and Psychotherapy at the University Clinic Ulm in November 2021. In addition, I would like to acknowledge the extension of my contract in 2025, which allowed me to focus on the preparation and completion of the manuscripts for this cumulative PhD thesis.

To the best supervisor I could have wished for: Prof. Stefan O. Reber, a very big and warm-hearted thank you goes to you. Even in difficult, exhausting and challenging phases of the experiments, you remained calm and found solutions for upcoming problems. Keep up your great, understanding and friendly manner so that future PhD candidates will be impressed by you as their boss, as I am. Thanks for the skills you taught me in the animal facility, your support during the long and hard experimental days and the memories we share together outside the lab.

Many thanks also goes to the members of my Thesis Advisory Committee, apl. Prof. Dr. Melanie Haffner-Luntzer, who reminded me that as a woman you should not put your own wishes on the back burner and that you can always pursue your own goals, and Prof. David A. Slattery for the successful collaboration, and the constructive inputs in our regular meetings. I wish him all the best. Moreover, I would like to express my special thanks to Prof. Christopher A. Lowry for his valuable input and expertise in supporting the preparation of microbial data and the manuscript of the *Molecular Psychiatry* paper, and for stepping in as my external supervisor at short notice.

Additionally, I want to thank the International Graduate School in Molecular Medicine for the opportunity to join the graduate program, the support during the entire period, not only in organization, but also enabling me to attend in conferences oversea and inside Germany.

A special thanks goes to Dr. Dominik Langgartner. You are calmness personified, your knowledge of your field of research is immense and impressive. Your career is outstanding, which makes you a role model for all young scientists at the beginning of their career. Stay as collegial and approachable as you have always been to me.

I would also like to thank Pei-Ling Tsai, without whose approval of the move to Ulm, your support and help during the entire phase, this major project would not have been as manageable as it was.

A big thank you goes to the two wonderful people who shared the office with me. Even though sometimes we could have been participants in our own *M. vaccae* studies; Tamara Schimmele and Giulia Mazzari – you made this long and intense journey much more bearable with our conversations. I would also like to thank Luke Desmond for the deep conversations we had, your support, the corrections, and inputs you gave me during the writing process of this thesis.

Great thanks goes as well to Petra Hornischer, Ulrike Binder and Milica Simov for their enormous patience in checking calculations and for their support during long experimental days, establishments and work in the lab. Thank you, Carmen Moll for all the organizational work you took over for me.

Thanks to all the other master and bachelor students choosing our working group to make first basic research experiences, especially Yorick Bailer, Antonia Struck and Sina Beck for supporting also the performance of all the experiments we did in the animal facility and the laboratory.

Finally, I would like to thank my parents for their continuous support over the years, especially during the final weeks of completing this thesis. I am deeply grateful for the opportunity they gave me to pursue my chosen path, as well as for their unconditional love.
Last but not least, I would like to express my deepest gratitude to my husband. You have been by my side throughout my PhD journey, from the very beginning of our relationship. Your constant support, patience, and encouragement mean more to me than words can express. I am truly grateful to have you and look forward to our shared future.

Thank you all.

Jessi

Statutory Declaration

I hereby declare that I wrote the present dissertation with the topic:

Investigation of the protective effects of *M. vaccae* ATCC 15483^{T} against the negative consequences of early life adversity or chronic psychosocial stress during adulthood.

independently and used no other aids that those cited. In each individual case, I have clearly identified the source of the passages that are taken word for word or paraphrased from other works.

I also hereby declare that I have carried out my scientific work according to the principle of good scientific practice in accordance with the current „Satzung der Universität Ulm zur Sicherung guter wissenschaftlicher Praxis" [Rules of the University Ulm for Assuring Good Scientific Practice].

Ulm, ……...

_____

Jessica Schiele